Contribute to Know the Enzymes that Secreted by *Trichoderma viride* and its Spores Loaded by Alginic Acid on Cucumber Seeds

Contribute to Know the Enzymes that Secreted by *Trichoderma viride* and its Spores Loaded by Alginic Acid on Cucumber Seeds

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Abstract. *Trichoderma* species are utilized as a biological fungicide in agriculture due to their great antagonistic ability. Additionally, they can increase nutrient uptake, fertility, stress resistance, and plant development. They are also opportunistic and symbiotic infections, which may cause plant defense systems to become active. There is a need to reveal the molecular underpinnings of the disease resistance that *Trichoderma* induces despite research documenting its biocontrol effectiveness against fungal diseases. Therefore, the objective of the current investigation was to identify the fungus-secreted enzymes and its spores loaded by alginic acid on cucumber seeds

Keywords. Alginic acid, Chitinase enzyme, Cucumber seeds, Lipase enzyme, Protease enzyme, *Trichoderma viride.*

1. Introduction

Biological control agent application is a promising and environmentally friendly strategy for enhancing present levels of agricultural productivity. By decreasing the usage of chemical pesticides, it helps to limit the discharge of their residues into the environment. Creating BCAs for disease control on their own or integrating them with lower chemical dosages to suppress phytopathogens is one of the most effective approaches to accomplish this goal [1]. This will have a little environmental impact. There are now a number of these agents that have been approved for use as commercial goods. These strains are from both bacterial and fungus genera, including *Agrobacterium*, *Pseudomonas*, *Streptomyces*, and *Bacillus*, as well as *Gliocladium*, *Trichoderma*, *Ampelomyces*, *Candida*, and *Coniothyrium*. Due to their capacity to produce significant quantities of enzymes and secondary metabolites for the management of phytopathogens, several species of *Trichoderma* are utilized in agriculture. *Trichoderma's* method of action includes mycoparasitism, the creation of specific enzymes that are antimicrobial, antibiosis, competition, and the induction of resistance in host plants. Chitinases, -glucanases, cellulases, and proteases make up the hydrolytic enzyme complex that the fungus of the genus *Trichoderma* generate. These enzymes are able to break down phytopathogens' cell walls, which facilitates hyphae penetration, colonization, and the development of myoparasitism. Chitinases are the most efficient of these enzymes for biologically controlling a variety of plant diseases and pests [2].

It is important to think about how to apply a microbe-based product to the plant in mass quantities, as well as the process of mass generating a biocontrol agent and developing a microbial formulation.

Previous studies have highlighted the importance of developing efficient and workable delivery mechanisms for the administration of biocontrol agents to the soil ecosystem as a key component of biocontrol technology [3]. A formulation is defined as a consistent and uniform mixture of inactive and active chemicals that yields an easy-to-use, risk-free, and highly effective treatment against the disease in question. Microbes are improved in many ways by the use of carrier materials, including but not limited to: portability; stability; efficacy; and ease of use in the field [4]. There are a wide variety of formulations that have been described. These include powders, granules, pellets, wettable powders, capsules or beads, water-dispersible granules, and emulsifiable liquids. The powder composition poses a health risk to the applicator and is difficult to weigh and administer in the field. Therefore, the objective of the current investigation was to identify the fungus-secreted enzymes and its spores loaded by alginic acid on cucumber seeds.

2. Methods and Materials

2.1. Loading T.viride on Alginic Acid

After a week of growth in PDA medium in a petri dish, T.viride was brought to the lab to be cleaned in sterile distilled water. T.viride loaded on alginic acid was prepared after spores were collected, counted using a hemocytometer, and then employed. At a concentration of 7.2 10^6 spores/mL, T.viride was present. T.viride spores were suspended in 50 mL of sterile sodium alginate solution (2% w/v) and gently agitated for 1 hour in a shaking incubator. To ensure that the alginate was dissolved evenly throughout the mixture, it was agitated vigorously. Next, 0.1 M $CaCl_2$ was added at room temperature and the mixture was extruded through a sterile syringe while being gently stirred [5]. To determine the health of the fungus, cucumber seeds were soaked for 1 minute, then placed in hydroxide until consolidated, washed with distilled water, dried on filter paper, placed at 10 ^oC for a day, and stored at room temperature for 6 months (as depicted in figure 1).

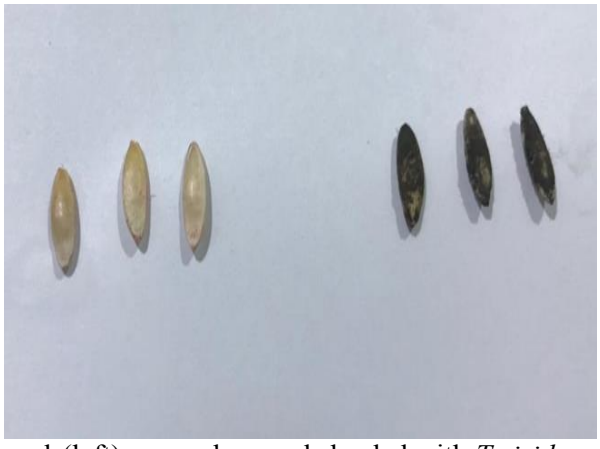

Figure 1. Cucumber seeds(left), cucumber seeds loaded with *T.viride* spores and alginic acid.

2.2. Testing the Effectiveness of the T.viride Spores Loaded with Alginic Against the Pathogenic Fungus Pythium Aphendermata.

Pots were taken and filled with sterilized peat moss. The following treatments were tested with three replications. Five seeds were sown for each pot, figure 2.

- Seeds loaded with *T.viride* (ST.V)
- Seeds contaminated with the *P.aphendermata* (SP.A)
- A culture medium contaminated with *T.viride* and a culture medium contaminated with the pathogenic fungus, *P.aphendermata.*(T.V + P.A)
- The control (C).

After 10 days, we determined what proportion of seeds germinated, and after 20 days, we determined what percentage of seedlings survived.

% Germination= (Number of germinated seeds)/(Number of total seeds)×100

%Death=(Number of dead seedlings)/(Number of germinating seedlings)×100

Figure 2. Experience treatments.

2.3. Enzymes Production

2.3.1. Lipase Activity

Using a spectrophotometric test based on the hydrolysis of pNP-palmitate at a concentration of 1.5 mmol L^{-1}, lipase activity was determined. A 50 mmolL^{-1} tris (hydromethyl) aminomethane (Tris) - HCl buffer (pH 8.0) containing 1 g L^{-1} gum arabic and 8 L of 15 mmol L^{-1} pNP-palmitate dissolved in 2-propanol made up the reaction mixture. After bringing the mixture back up to 30 degrees Celsius, 20 L of enzyme solution was added. After 5 minutes of incubation at 30 degrees Celsius, 0.3 millilitres (mL) of Marmur solution (chloroform: isoamyl alcohol, 24:1) were added to halt the reaction [6]. The material was centrifuged for 5 minutes at 4 degrees Celsius and 10,000 rpm to separate the aqueous supernatant. Next, 410 nm was used to determine the density of the supernatant.

2.3.2. Chitinase Activity

An test for chitinase activity was conducted following Miller's guidelines [7]. One millilitre of crude enzyme solution and one millilitre of 0.5% colloidal chitin in 0.1 millilitres of citrate buffer (pH 6) were mixed and incubated for 30 minutes at 37 degrees Celsius in a water bath with constant shaking. In order to halt the reaction, 2 mL of DNS reagent was added. The dye was given a 5-minute soak in hot water. At 575 nm, we found that a blank made up of 1 mL of a 0.5% substrate-buffer solution, 1 mL of buffer, and 2 mL of DNS gave the most accurate reading.

2.3.3. Protease Activity

To determine the protease activity we used the method of Soad [8]. The reaction media is 2.5 ml in volume and contains 100 mM phosphate buffer (pH 6), 1% soluble casein, and an enzyme source (culture supernatants). After 30 minutes of incubation at 40 degrees Celsius, 2 millilitres of trichloroacetic acid (at a concentration of 15 percent) were added to the reaction mixture.Protein precipitate was removed by centrifuging the reaction mixture at 5,000 rpm for 10 minutes.Half a millilitre of the resultant supernatants was pipetted into a test tube with 1.5 millilitres of 0.33 M sodium hydroxide. At a wavelength of 280 nm, the absorbance of the supernatant was measured [9], period(s) of development. Under these conditions, one unit of protease activity was defined as the amount of enzyme necessary to liberate one mole of tyrosine per minute.

3. Results and Discussion

3.1. The Effect of T.viride Spores Loaded with Alginic Acid on Pathogenic Fungus on Cucumber Seeds in Pots in the Laboratory

Results in the table (1) shown that the highest percentage of germination was in the treatment ST.V reaching to 100, followed by treatment T.V + P.A. amounting 66.66, it was with a significant differences from the rest of the treatments, the lowest percentage of germination by the impact of SP.A which reached 53.66.

While the results of dead seedlings, table (1), showed a highest ratio of dead in SP.A treatment which reached to 60.33 which differed significantly from the rest treatments following by treatments T.V + P.A to 20.50. The lowest ratio of dead seedlings was in ST.V and control reached to 0.

Table 1. The effect of *T.viride* spores loaded with alginic acid on pathogenic fungus on germination, dead seedlings and the severity of infection in cucumber seeds.

Treatment	% Germination*	% Dead seedlings*
ST.V	100.00	0.00
SP.A	53.66	60.33
T.V + P.A	66.66	20.50
C	96.33	0.00
$L.S.D_{0.05}$	0.43	12.10

* The value represent in triplicate.

3.2. Enzymes Production

The results showed that *T.viride* can produce lipase enzyme, figure 3, and chitinase enzyme, figure 4, were in the highest concentration 0.527 U/mL and 0.454µmol/min respectively after an incubation period of 15 days, while the highest concentration of protease were after an incubation period of 30 days which was equl to 0.357 mol/mL figure 5.

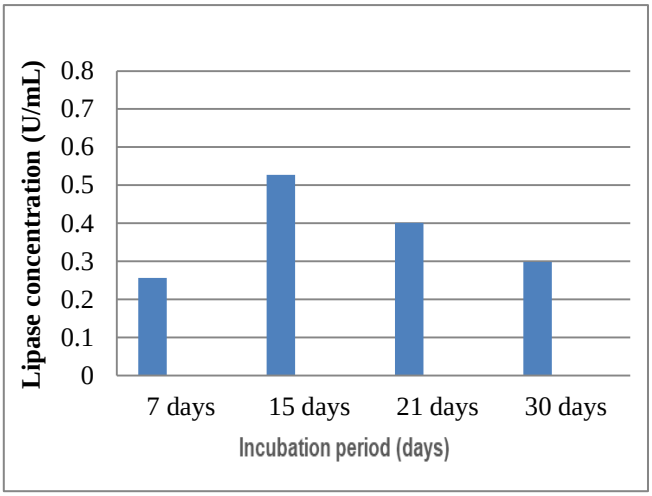

Figure 3. Lipase concentration in culture medium of *T.viride* in an incubation period of 7, 15, 21 and 30 days.

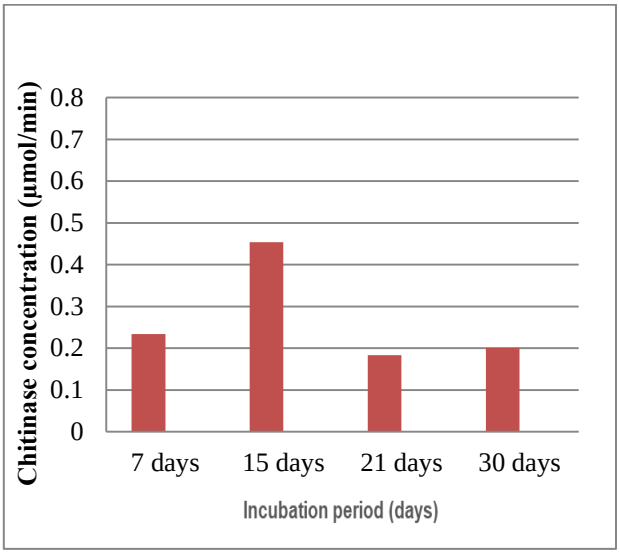

Figure 4. Chitinase concentration in culture medium of *T.viride* in an incubation period of 7, 15, 21 and 30 days.

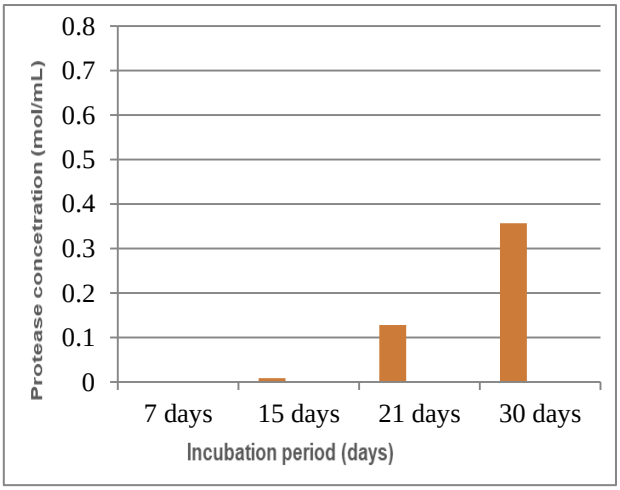

Figure 5. Protease concentration in culture medium of *T.viride* in an incubation period of 7, 15, 21 and 30 days.

Recent biological control research has focused on the fungus *Trichoderma*, which is made up of a variety of fungi that function as biological control agents. These fungi's antagonistic traits are dependent on the activation of several processes. One of these methods is the creation of certain substances and metabolites, such plant growth hormones, hydrolytic enzymes, and the release of enzymes that degraded the cell wall, like chitinase, protease, glucanase, and others. These enzymes prevent the growth of pathogen spores, enzymes that hydrolyze fungal walls, toxic secretions, and hydrolyzed hyphae [10]. Biological control refers to the practise of employing beneficial organisms, their genes, and/or their byproducts, such as metabolites, to mitigate the harmful effects of plant diseases and stimulate good responses in the plant. Disease suppression is mediated by biocontrol agents and is the consequence of interactions between the plant, the pathogens, and the microbial population. One of the most commonly found and isolated soil-borne enemies is the fungus Trichoderma. Due to their ability to defend plants and contain pathogen populations in a wide range of soil conditions, these fungi have been the subject of intensive research and commercial marketing as biopesticides, biofertilizers, and soil additives. Secondary metabolites and cell-wall degrading enzymes are just two examples of the physiologically active compounds produced by Trichoderma spp. The processes of partner identification and cross-talk that perpetuate the beneficial association between Trichoderma and the plant and the pathogen are the focus of current research into this triadic

interaction. To learn more about this complex triadic interaction, researchers have used techniques including genomes, proteomics, and, more recently, metabolomics [11].
The fungicide bio vaccine protects plants against rot and wilt diseases by containing *T.viride*. It eliminates fungus-based pathogens including *Pythium*, *Rhizoctonia*, and *Fusarium* species that cause a variety of rot and wilt illnesses. *T.viride* forms coils around pathogens and produces different enzymes including cellulose and chitinases to break down the cell wall production of fungi. This method of killing other fungi by inhibiting their development and metabolic activity is known as mycoparasitism. Additionally, it strengthens systemic resistance to eradicate plant pathogens. It promotes stress tolerance while also enhancing nutrients and moisture in the root system [12]. Cell wall-degrading enzymes such cellulase, xylanase, pectinase, glucanase, lipase, amylase, arabinase, and protease can be produced by *Trichoderma* species [13]. Because they contain chitin, cellulose, clogan, and proteins, these enzymes are crucial in the degrading of the cell walls of pathogenic fungi [14,15]. The isolation and purification of enzymes require more research, even though the current work provides a general understanding of the *T.viride* and its capacity to produce enzymes. The potential for the fungal isolates to manufacture enzymes for industrial use is quite high.

Conclusion

In conclusion, our results showed the inhibitory effect of *Trichoderma spp* represented by *T.viride* against *P.aphendermata* by producing extracellular enzymes that may affect the functioning of *P.aphendermata* and the protection of seedlings and seeds by loading them by fungal spores.

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