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TRICHODERMA SPP. SOURCE OF HYDROLASE ENZYMES WITH ROLE IN *F. OXYSPORUM* INHIBITION

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Abstract

The fungi from Trichoderma genus are a very large group of microorganisms that are present in nearly all agricultural soils and play a significant role in plant protection. Enzymes produced by species of genus Trichoderma constitute an important group of biotechnologically enzymes because of the versatility of its properties and ease of mass production. This study was focused on the effect different factors of antagonistic T. harzianum (ICCF 417) and T. koningii (ICCF 418) against pathogenic fungus F. oxysporum (ZUM 2407) by microbiologic and biochemical tests. There was different patterns in cell wall degrading enzymes production by Trichoderma isolates so production of chitinase was 113.93 % by T. harzianum comparative to T. koningii, the lipase was 38.53% in T.harzianum comparative to T. koningii while the protease was 91.43% in T. harzianum comparative to T. koningii. Both Trichoderma strains studied showed maximum lipase activity at pH 9, which was significantly higher than those at other pH levels. On the other hand, optimum pH for producing highest activity of protease was pH 6. A high level of chitinase activity was observed in the culture medium with pH 6. Our results showed that hydrolase activities studied in this experiment play an important role in pathogenic fungus F. oxysporum inhibition and the degree of effect is different.

Key words*: Trichoderma harzianum, Trichoderma koningii, lipase, protease.*

INTRODUCTION

The use of *Trichoderma* fungus species against many fungal phytopathogens has seen significant progress in recent years because their high reproductive capacity, ability to survive under very unfavorable conditions, efficiency in the utilization of nutrients, strong aggressiveness and efficiency in defense mechanism (Harman, 2006; Harman et al., 2004). Due to their ability to protect plants and because they are commonly found in almost all soil types, these fungi have been widely studied and commercially marketed as biopesticides, biofertilizers and soil amendments. *Trichoderma* spp. were shown to be very efficient producers of extracellular enzymes like chitinases, β-1.3-glucanases, cellulases, lipases, proteases some of these have been implicated in antifungal effect on different pathogens (Gajera et al, 2012; Vinale et al., 2007; Harman, 2006; Markovich and Kononova, 2003; Viterbo et al., 2002; Monte, 2001). Several reports have been given to explain the role of lytic enzymes produced by *Trichoderma* spp. during plant defense. *Fusarium oxysporum*, a well-known species of soilborne fungus, causes significant losses to horticultural and ornamental crops throughout the temperate climatic regions (Chen et al., 2017). The fungus *Fusarium* spp. is a fungal species that have adapted to different range of environmental from worldwide, in addition this types was described as the important pathogen of the different crops. Lobna et al. (2017) and Houssein et al (2010) found that the use of the *T. harzianum* as strategy to improve the response of the tomato resistance against

Fusarium wilt under greenhouse conditions caused biochemical changes in plants. The correlation between the enzyme level and resistance to the pathogens was increased, especially under conditions of stress and attack pathogens. Enzymes activity plays an important role in plant resistance against attacking pathogens (Zhang et al., 2008; Cherif et al., 2007; Mohammadi and Karr, 2002). The inhibitory effect of *Trichoderma* on the phytopathogenic *F. oxysporum* could be due to the secretion of lipolytic and proteolytic enzymes that inactivate enzyme of *F. oxysporum* thereby decreasing its pathogenity (Elad and Kapat, 1999).

The aim of this study was the evaluation of different factors from live and dead cells of *T. harzianum*, (ICCF 417) and *T. koningii* strain (ICCF 418) on hidrolytic enzyme production that influence virulence against pathogenic fungus *F. oxysporum* strain (ZUM 2407).

MATERIALS AND METHODS

The ability of fungi *T. koningii* and *T. harzianum* to produce enzymes were evaluate in synthetic medium. The fungus was grown in 250 ml Erlenmeyer flask that contained 100 ml of synthetic medium (SM) then we add the specific substrates for each enzyme separately, then cultures were incubated at 28°C and the ability of enzymes production was determined after 1, 2, 3 and 4 weeks of incubation. Also enzymes were used diluted to 100%, 50% and 10%. The fungi weight was determined by weighting an empty tube, then put (1 mL) of the fungi from each period of culture into the weighted tube after that drying and centrifuging and taking the precipitate which was dried at 50^0C for 3 days, then weighted again. Preparation of cell lysate was done by taking 10 ml of each culture separately and centrifuged. The precipitate obtained was re-suspended in 5 mL of phosphate buffer (pH 7.4) with vortex, then adding sand to the tube, put it in IKA® ULTRA TURRAX device at 6000 rpm for 30 s then put it at ice for 1 min., replay for three times and centrifuged at 10000 rpm for 10 min. at 4°C. Then the solution obtained filtered by Millipore 0.22 µm, and aliquots of the supernatant were used for next assays. All cultures were put at 100^0C for 15 min., filtered by micro filter, aliquots of the supernatant were used for next assays. Characterization of enzymes produced by *T. koningii* and *T. harzianum* that had effect on *F. oxysporum* was done in PDA medium using a Petri dishes divided into 6 sections. At the first one we put 10 µL synthetic medium SM, the second 10 µL of dead cell of enzymes culture, the third one 10 µL of living cell of enzymes culture, the forth section 10 µL of dead cell from fungi culture, the fifth with 10 µL of dead lysate, the last one with 10 µL living lysate.

For control, we put a Petri dish of *T. koningii*, *T. harzianum* and *F. oxysporum* alone and a Petri dish divided into two parts, one with *Trichoderma* and the other with *F. oxysporum.* All experiments were made in triplicate.

Chitinase activity assay was performed according to Miller (1959). Lipase activity was measured spectrophotometrically using an assay based on the hydrolysis of p-nitrophenyl palmitate as substrate, according to Gupta et al. (2002) while for determination of protease activity has been used the method of Saad (1995). The protein quantity of the crude enzyme extract was determined by the Lowry method using bovine serum albumin as standard (Lowry et al., 1951). The enzymes activity and protein concentration were measured after 1, 2, 3 and 4 weeks incubation of the culture incubation. In order to determine the optimum pH value for the enzyme produced by *Trichoderma* obtained after fermentation, the activity of the enzyme was assayed between the pH values of 3.0-9.0.

RESULTS AND DISCUSSIONS

Figure 1 shows the influence of pH on chitinase, lipase and protease production by *T. harzianum*, *T. koningii* and *F. oxysporum*. According to the results, the optimal pH for lipase was 9. The results are similar with Ulker et al., 2014, which showed the optimum pH value for lipase activity produced by *T. harzianum* was 8.5. Also has been establish that optimum pH for maximum chitinase and protease activity in *T. harzianum* and *T. koningii* was pH = 6. This agrees with results obtained by Cirano et al., 1991, and Kredics et al., 2004.

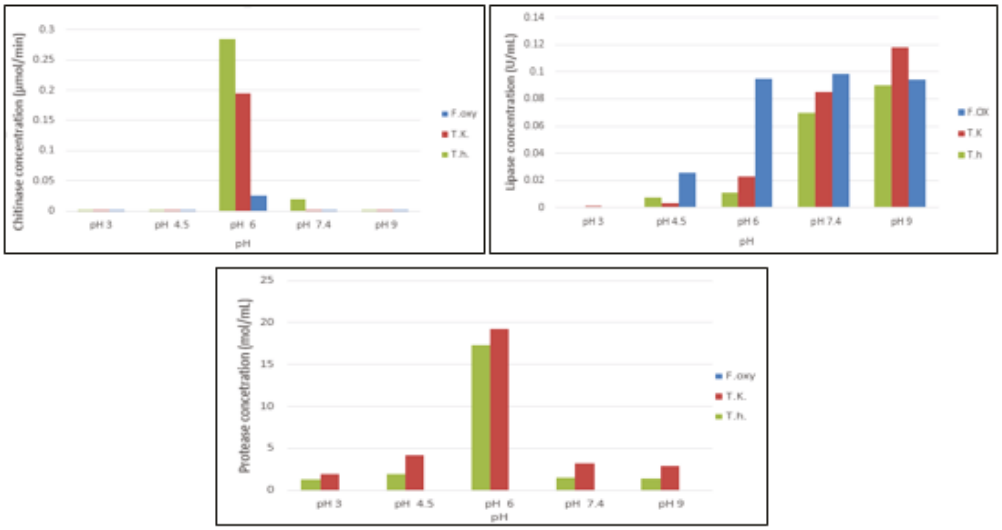

Figure 1. Determination of optimum pH for chitinase, lipase and protease enzymes production by *T. harzianum*, *T. koningii* and *F. oxysporum*

The results of chitinase enzyme showed that the two fungi can produce the chitinase but its level on *T. harzianum* was higher than *T. koningii*, and for the two fungi the period of 14 days was the highest production, also the dilution ratio 100% was the highest, as shown in figure 2.

The levels of chitinase enzyme was 0.42 and 0.48 µmol/min in *T. koningii* and *T. harzianum*, respectively, after 14 days.

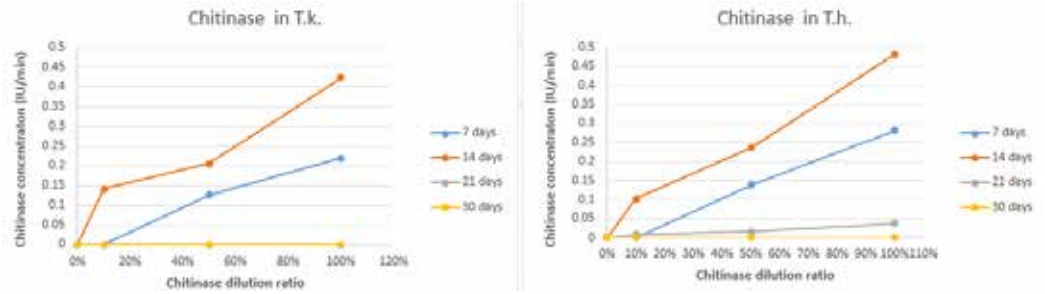

Figure 2. Chitinase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The production of lipase enzyme in synthetic medium for *T. koningii* was higher than *T. harzianum* and the second week was the highest period of production, as shown in figure 3.

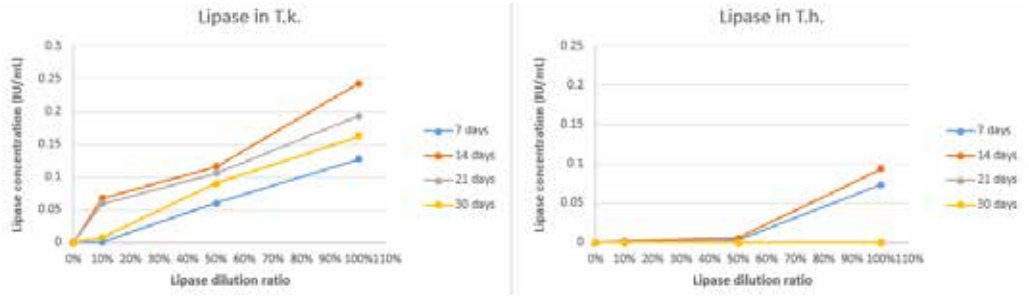

Figure 3. Lipase production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The results of protease production by *T. koningii* and *T. harzianum* are presented in figure 4. The protease levels in *T. koningii* was higher than in *T. harzianum*, and the 30 days incubation period was with the highest production, also the dilution ratio 100% was the highest increasing.

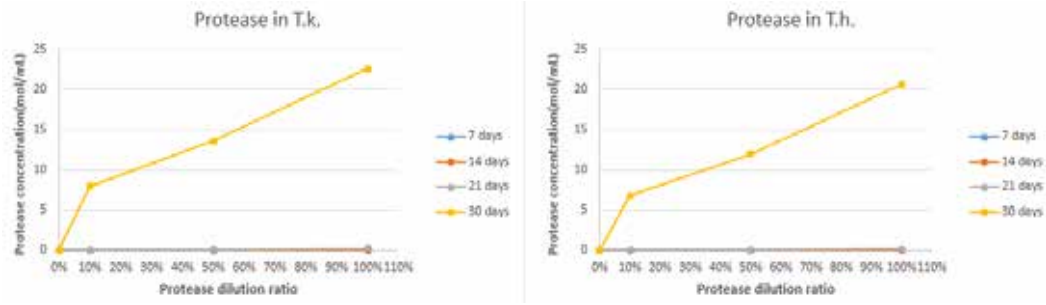

Figure 4. Protease production in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The protein levels in chitinase enzyme were in *T. harzianum* higher than the *T. koningii*, and the second week had the highest levels of protein in the two fungi, as shown in figure 5.

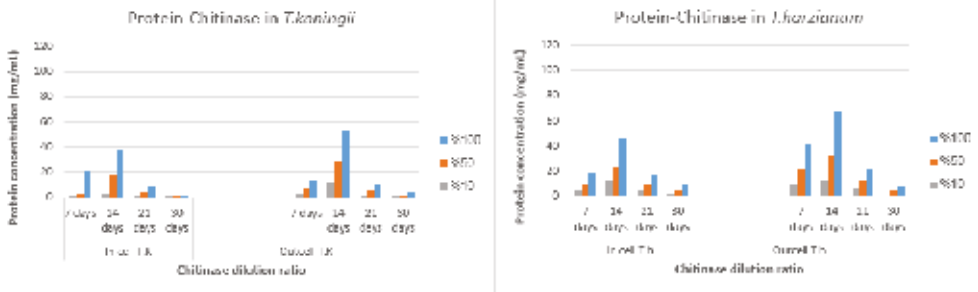

Figure 5. Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The highest protein level in the lipase enzyme medium was in *T. koningii* in comparison with its level in *T. harzianum*, but also the period of 14 days was the highest level of protein production in comparison with the rest periods, as shown in figure 6.

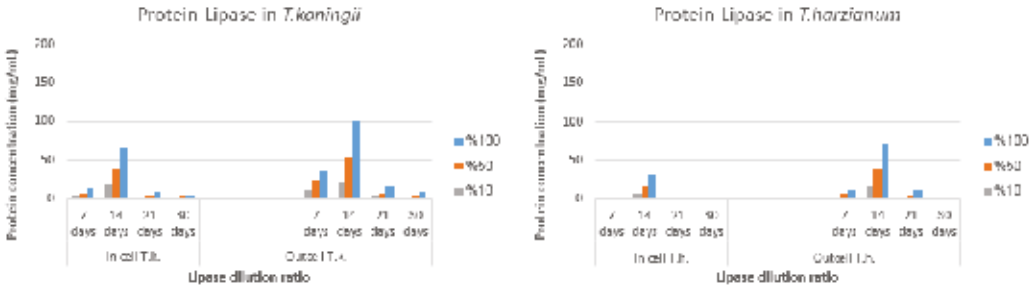

Figure 6**.** Protein levels in *T. koningii* and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

In figure 7 are presented the levels of proteins in synthetic medium for protease production by in *T. koningii* and *T. harzianum* respectively, protein levels on *T. koningii* was higher than *T. harzianum*, and for the two fungi the period of 30 days was the highest production.

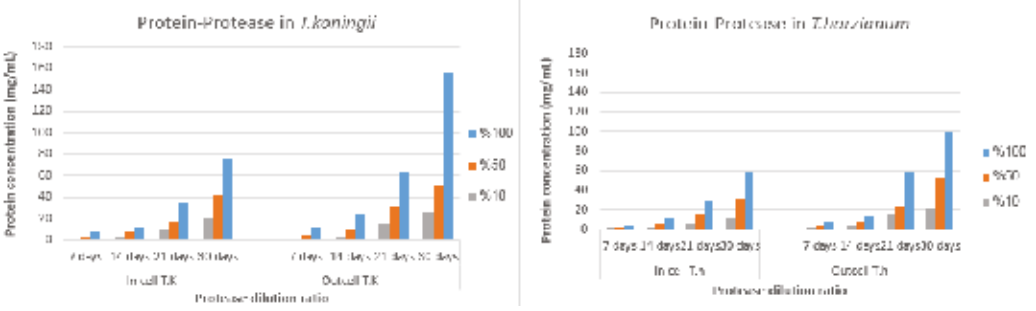

Figure. 7. Protein levels in *T. koning*ii and *T. harzianum* in a different dilution ratio of enzyme and for a period 1, 2, 3 and 4 weeks

The levels of protein in *T. koningii* were 52.97, 101.85 and 155.23 mg/mL for chitinase, lipase and protease, respectively, in out of cell, while in cell lysate were in chitinase 36.98 mg/mL, lipase 65.69 mg/mL and protease 75.68 mg/mL. In *T. harzianum* the protein levels were 67.85, 72.02 and 99.25 mg/mL for chitinase, lipase and protease, respectively, in out of cell, while in cell lysate were in chitinase 45.26 mg/mL, lipase 31.68 mg/mL and protease 58.61 mg/mL. The results of the effect of *T. harzianum* and *T. koningii* on *F. oxysporum* showed that these fungi have a high ability against the pathogenic fungi *F. oxysporum*, the ratio reached to 1, according to the scale of Bell et al. (1982). The lipase and the protease enzymes were effective on *F. oxysporum* in the second and forth weeks, respectively. These results are in accordance with those obtained by Elad et Kapat, 1999, which supported that some proteases and lipases secreted by *Trichoderma* spp. may be involved in inactivating extracellular enzymes of *F. oxysporum*.

CONCLUSIONS

The results obtained in this study show that both strains of *Trichoderma* studied produced chitinases, proteases and lipases in synthetic medium but the concentration depends on the strain and the incubation period.

There is an inhibitory effect of *T. harzianum*, strain ICCF 417 and *T. koningii* strain ICCF 418 against pathogen *F. oxysporum* strain ZUM 2407 by production of protein and extracellular enzymes which may effect on the action *F. oxysporum*.

The lipase has the highest weight in comparison with the others enzymes, while the chitinase enzyme was not effective on *F. oxysporum* for all period of incubation.

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