SUMMARY

The PhD thesis titled " ***CONTRIBUTIONS TO STUDY THE USE OF TRICHODERMA HARZIANUM AND T. KONINGII FUNGI TO CONTROL THE SOIL PATHOGENS, THEIR ENZYMATIC CHARACTERIZATION AND THE PREPARATION OF BIOFUNGICIDE" elaborated by PhD. MOHAMMED NAITHEL RADHI, Scientific coordinator Prof. univ. PhD. Ioan ROŞCA, 2018,USAMV, Bucharest***

KEY WORDS: Biological control, soil pathogens, biopesticidal characters, *Trichoderma harzianum*, *T. koningii*, enzyme production, tomatoes

A microbiological and biological study of bio-fungi *T. harzianum* and *T. koningii* was conducted which included study the effect of enzymes production and filtration of this bio-fungi, as well as study of the effect of the loading of bio-fungal spores on alginic acid and 2% CMC against pathogenic fungi *F. oxysporum*, *R. solani* and *Pythium spp*. in laboratory and field on tomato plant. The results showed the ability of bio-fungi *T. harzianum* and *T. koningii* to inhibit the growth of pathogenic fungi *F. oxysporum*, *R. solani* and *Pythium spp*. by dual culture process, the antagonistic ratio reached to 1 in *F. oxysporum* and *Pythium spp*, while against *R. solani* the antagonistic ratio reached to 2 according to the Bell scale.

While the results of fungi growth in liquid malt extract medium after 12 hours of fungi culture, *F. oxysporium* where it reached to the highest growth (0.42) of the fungus followed by fungi *Pythium spp.* and *T. harzianum* (0.37) and (0.33) respectively, for *T. koningii* and *R. solani* the growth reached to (0.31) and (0.30) respectively, also the fungi growth in liquid malt extract medium after 72 hours from culture, *T. koningii* where it reached to the highest growth (1.30) of the fungus followed by fungus us *T.harzianum*, *F. oxysporium*, *Pythium spp.* and *R. solani* which reached (0.95, 0.87, 0.17 and 0.14) respectively.

The study showed the ability of bio-fungi *T. harzianum* and *T. koningii* to produce of enzymes cellulase, amylase, chitinase, lipase and protease. The halo diameter formed on the solid medium for cellulase reached to 5.75 cm and 6.5 cm for *T. harzianum* and *T. koningii* respectively, the production the amylase was very low which reached to 1 cm in *T. harzianum* and 0.75 cm in *T. koningii*. The chitinase production was 5.25 cm in *T. harzianum* and 6.25 cm in *T. koningii*. For the lipase production a white crystals immersed in the medium around the colony was appear for *T. harzianum* and *T. koningii*. The halo diameter formed on the solid medium for protease reached to 1.7 cm and 2.6 cm for *T. harzianum* and *T. koningii* respectively.

Also the results show the ability of fungi *T. harzianum* and *T. koningii* to dissolve the phosphorus in solid medium, where the halo diameter was 1.8 cm and 2.5 for fungi *T. harzianum* and *T. koningii* respectively.

The results of bio-fungi filtrates showed their inhibitory ability against pathogenic fungi in the petri dish on PDA medium in a different concentrations where the highest inhibition was when using 30% bio-fungi filtrates. Also the results showed that bio-fungi *T. harzianum* and *T. koningii* were able to produce enzymes in the potato dextrose broth (PDB) after one week of fungi culture, it can be observed that the highest production of enzymes was in chitinase enzyme (0.1549), while the lowest production of enzymes was in amylase enzyme (0.000125). While the enzymes production for *T. koningii* cultured in PDB showed the highest production of enzymes was in chitinase enzyme (0.09985), while the lowest production of enzymes was in amylase enzyme (0.000482).The results of optimal pH for enzymes production in *T. harzianum* and *T. koningii* showed that the highest production was at pH = 6 for FPase, CMC, cellobiase, amylase, chitinase, protease. While the highest production for lipase enzyme in *T. harzianum* and *T. koningii* was at pH = 9.

The study also showed the ability of fungi *T. harzianum* and *T. koningii* to produce enzymes efficiently in the induced medium. The results of FPase enzyme showed that the two fungi can produce the FPase but its level on *T. koningii* was higher than *T.harzianum*, and for the two fungi the period of 14 days was the highest production, which reached to (0.244 and 0.078 µmol/mL/min) in *T. koningii* and *T. harzianum* respectively. Also the production of CMCase enzyme in synthetic medium in *T. koningii* was (0.260 IU/mL/min) and it was higher than *T. harzianum* which reached to (0.095 IU/mL/min) and the second week was the highest period of production, cellobiase levels on *T. koningii* was higher than *T.harzianum*, and for the two fungi the period of 14 days was the highest production, which reached to (0.192 and 0.130 IU/mL/min) in *T. koningii* and *T. harzianum* respectively. The production of amylase enzyme in synthetic medium in *T. koningii* = 0.336 µmol /µmol maltose / hour was higher than *T. harzianum* = 0.284 µmol/mg maltose / hour and the second week was the highest period of production. 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, the levels of chitinase enzyme was 0.422 and 0.481 U/min in *T. koningii* and *T. harzianum* respectively. The production of Lipase enzyme in synthetic medium in *T. koningii* = 0.242 IU/mL was higher than *T. harzianum* = 0.093 IU/mL and the second week was the highest period of production, protease levels on *T. koningii* was higher than *T. harzianum*, and the 30 days incubation period was with the highest production. The levels of protease enzyme was 22.57 and 20.64 U/mg protein in *T. koningii* and *T. harzianum* respectively. The results of mycelium weight

showed that the weight for second and third weeks were higher than the first and forth weeks, also the CMCase was the highest weight in comparison with the others enzymes. The protein levels for all enzymes produced by *T. koningii* and *T. harzianum* were in out cells higher than in cells. Also the results of characterization of enzymes that produced from *T. koningii* and *T. harzianum* that effect on pathogenic fungi *F.oxysporum*, *R. solani* and *Pythium spp.* in PDA medium showed the effect of some enzymes on these pathogenic fungi.

The results of the effect of fungi *T. harzianum* and *T. koningii* and their filtrates on pathogenic fungi (*F.oxysporum*, *R. solani* and *Pythium sp.)* on tomato plant in pots in the laboratory showed that the highest percentage of germination was in the treatment *T. koningii* and *T. harzianum* reaching to 100% and 93.33% respectively, the lowest percentage of germination by the impact of pathogenic fungi was *R. solani* which reached 53.33%. While the results of dead seedlings showed a highest ratio of dead in *R. solani* treatment which reached to 37.5%. The lowest ratio of dead seedlings was in *T.harzianum*, *T. koningii*, extract of *T.harzianum*, extract of *T. koningii* and control reached to 0%. The results of infection severity also shown a highest ratio of infection severity in *R. solani* and *F.oxysporum* treatment which reached to 60% and 50% respectively, the lowest ratio of infection severity was in *T.harzianum*, *T. koningii*, extract of *T.harzianum*, extract of *T. koningii* and control reached to 0%. The results of plant growth indictors which revealed a highest ratio of length of leaf in *T. harzianum* treatment which reached to 16.66 cm followed by *T. koningii* treatment reached to 14.33 cm. for the leaflet's width the results of treatments *T. harzianum* 3.33 cm and *T. koningii* 3.16 cm were the highest. Results of root's length were as the following, the highest root's length were in *T. koningii* and *T. harzianum* treatments which reached to 14.0 and 13.5 cm respectively. The results of leaf's number showed a high ratio of leaf's number in *T. koningii* and *T. harzianum* treatments which reached to 6.33 and 5.66 respectively. The results of leaflet's number show a highest leaflet's number was in *T. harzianum* and *T. koningii* treatments which were 9.33 for *T. harzianum* and 8.33 for *T. koningii*. The highest percentage of phosphor was in the treatment *T. harzianum* and *T. koningii* reaching to 0.46 and 0.45 mg/Kg respectively, the highest chlorophyll levels in chlorophyll a (C_a), chlorophyll b ($C_{b)}$ and chlorophyll of xanthophyll (x)plus carotenoids (c), (C_{x+c}) were in extract of *T. harzianum* and extract of *T. koningii* which reached to 8.87 and 8.04 for C_a and respectively for C_b were 3.42 and 3.00 for in extract of *T. harzianum* and extract of *T. koningii* respectively and for C_{x+c} the level in extract of *T. harzianum* and extract of *T. koningii* which reached to 585.63 and 532.65 respectively.

The results of the effect of bio-fungi loaded on alginic acid and 2% CMC on the percentage of germination and seedling death in pots in the glass house showed the ability of bio-fungi loaded on 2% CMC and alginic acid but the effect of 2% CMC was higher than the bio-fungi loaded on alginic acid. The results of the field experiment were complemented by results of pots in the glass

house where the treatments were proved the ability of bio-fungi loaded on alginic acid and 2% CMC to protect the plant from the pathogenic fungi, treatments which containing alginic acid had a significant effect on the increase height and diameter, fruit weight, dry and soft root weight, dry and soft vegetable group weight, chlorophyll, nitrogen, phosphor, respiration and photosynthesis and decrease the severity of injury of tomato plant.