The role of *T. viride* in deterring *F.roseum* aggressiveness on maize seedlings

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Abstract- The direct confrontation between *T. viride* and the various fungi tested *in-vitro* showed that it had a high capacity for competition, either by invading the entire surface of the petri dish, or by stopping the growth of pathogenic fungi at a distance. When inoculating maize plants with a concentration of 10^5 spores / ml by *F.roseum* by spraying the aerial parts, after 14 days of infection, the symptoms of the disease appeared on most maize plants, as did the length of the roots and the measurements of all the vegetative parts decreased significantly compared to control plants. *T. viride* was tested against *F.roseum* where *T. viride* was treated with a concentration of 10^6 spores / ml.

After 22 days of treatment, the symptoms of the disease were disappeared, and the measures of root length and aerial parts were close to those of the control. It is an effective fungus against phytopathogens because it secretes several enzymes that degrade fungal or bacterial cells through the use of parasitism mechanism.

Key words: *F.roseum*, *T. viride*, maize seedlings, growth degradation, biological control.

1-Introduction

The corn plant is of great agricultural and biotechnological importance (conversion of the product into bioethanol). It was also considered the first cereal plant, which was changed in its genotype GMO. However, this plant remains vulnerable to many abiotic factors (drought, pollution of the oceans .....) and biotic factors (harmful insects and fungi ...). Abiotic factors influence the development of the maize plant, facilitating its infection by various fungal pathogens and bacteria (Ristanovic, 2001). These diseases also lead to loss of yield of the nutritional and commercial value of dry matter loss or inability to germinate, analysis of sugars, fats and proteins. As research a progress, another method of control has been discovered, biological control, this is normally present in ecosystems that rely on the surveillance of pathogens by other organisms, including arthropods, nematodes (Lee and Lee, 2007). For this reason, this study was conducted to demonstrate the ability of the fungus *Trichoderma viride* to inhibit the growth of phytopathogenic fungi.

2-Materials and Methods

2.1-Preparation of corn seedlings

Under good sterilization conditions, corn kernels were put in a petri dish containing hypochlorite of sodium 10% for two minutes, in order to sterilize the surface to remove both microbes and the pesticide used when treating the seeds. The latter have been dried by being placed inside a sterile filter paper, their transferred to petri dishes containing sterile filter paper saturated with sterile physiological water. Twelve grains were put systematically in the dish on the filter paper surface. After that, the grains were covered with another paper saturated with moisture. The dishes were incubated at a temperature of 22°c for 7 days (Benhamou and *al*., 1997).

After germination, the germinated seeds were transferred into pots containing 50 g of sterile soil and humus, where 10 seeds were put in earth pot. Twelve pots were prepared to realize the experiment, and five replications were made as control experiment. The pots were placed under normal conditions of lighting, ventilation and temperature (25-28°c) according to the weather conditions of May and June (2011). The seedlings were regulatory watered by plain water twice a week with about 50 ml for each pot once a week (Knop, 1965).

2.2-Inoculating the seedlings by pathogenic fungus *Fusarium roseum*

2.2.1-Preparing the sporal solution of *F.roseum*

Under good sterilization conditions, the sporal solution of *F.roseum* fungus was prepared by adding 5 ml of sterile distilled water to the fungal colony and then scraping the surface with an inoculation needle having the L shape; in order to obtain inicial solution of the sporal solution of *F.roseum*. After that, decimal dilutions (10^{-3}, 10^{-2}, and 10^{-1}) were prepared. After the preparation of the sporal solution, the spores of *F.roseum* were calculated using Thoma slice to obtain the concentration of 10^5 spore/ ml, and then the seedlings were inoculated (Gnancadja,2002).

2.2.2-Spraying the vegetative total with the suspension sporal of pathogenic fungi:

During the 04-leaf stage of maize seedling development, their leaves were sprayed with 50 ml of the sporal suspension of *F. roseum* at a concentration of 10^5 spores / ml (Woo and *al*., 2006).

2.2.3-Through the preparation of the sporal solution of the *Trichoderma viride*

The sporal solution of *T.viride* had been prepared in the same way that sporal fungal solution of *F.roseum* was prepared with an average of 10^6 spore/ ml and kept in the refrigerator till use (Rojan, 2010).

2.2.4-Treatment of maize plants by spraying the aerial part with the *T. viride* spore solution

After the onset of disease symptoms, the infected maize seedlings were sprayed with 50 ml of the sporal solution of *T. viride* at a concentration of 10^6 spores / ml (Windham and *al*., 1986).

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3- Results

3.1- Inoculation of corn plants with *F.roseum* by spraying the aerial parts

After 14 days of inoculation of maize seedlings, record a clear difference in growth and morphology of the infected plants for the three samples tested compared to the control plants. On the other hand, the appearance of the red color on the limb of the tested plant leaves, the latter have been wrapped and take the pale yellow color (figure 1et 2). Statistical analysis shows at the level of 95% and 2 degrees of freedom that the tested plants differ from those of the control plants by the calculated value of (0, 03-0.,003-0,029- 0,025) for each of the (roots, stems, leaves, and the distance between the nodes), respectively, and are less than 0.05. Therefore, there is a difference between the average length of the plant parts of the tested samples and the control sample is different from zero, indicating the effect of *F.roseum* on the growth of maize plants. The root length of the infected plant samples (1 - 2 - 3) was (13 – 8, 33 - 10) cm. The length of the stems was estimated at (13-08- 07) cm, while the length of the limb was (23-17-17) cm finally, the distance between the nodes was estimated at (1, 81-2, 21 and 2, 28) cm respectively. The control plants reached a total root length of 19 cm, 20 cm legs, 55 cm leaves and finally the distance between the contractures of 4.75cm (table1), (figure 3, 4).

Table 1: Length of roots and aerial parts (cm) of maize plants after 14 days of *F.roseum* infection

plants parts / The samples	Total root length (cm)	Length of stems (cm)	Length of leaves (cm)	Distance between nodes (cm)
Control	19	20	55	4,75
Sample 1	13	13	23	1,81
Sample 2	08,33	08	17	2,21
Sample 3	10	07	17	2,28

Figure1: Growth comparison of control plants with corn plants inoculated with *F.roseum* after 14 days of aerial spraying infection ((a) *F.roseum* infected plants, (b): control plants).

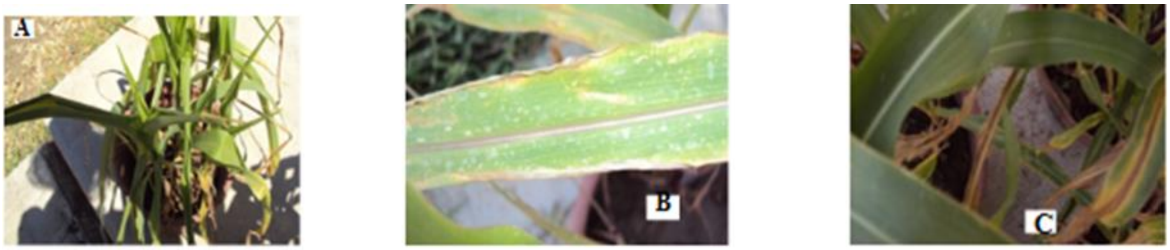

Figure2: Manifestation of the symptoms of *F.roseum* on the aerial parts, disappearance of the green color of the plants tested after 14 days of infection, ((a): disappearance of the green color of the leaves and lack of expansion of the blade with leaf curling, (b): yellowing of leaf margins with irregular white spots on the surface of some leaves, (c): burns in the center of the main leaf vein).

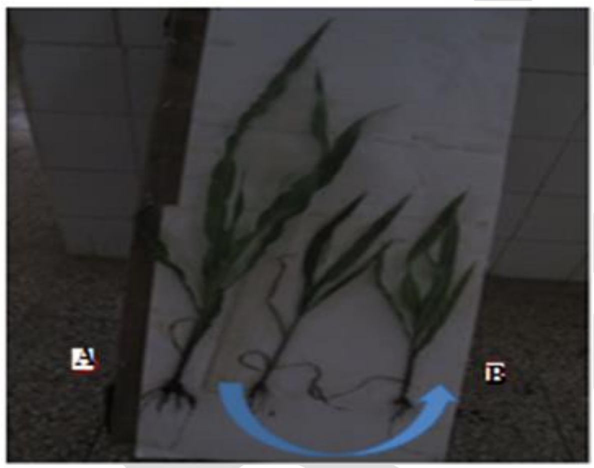

Figure3: Comparison of the length of the roots and stems of the control plants with those inoculated with *F.roseum* after 14 days of infection by aerial spraying ((a): plants infected with *F.roseum*, (b): control plants).

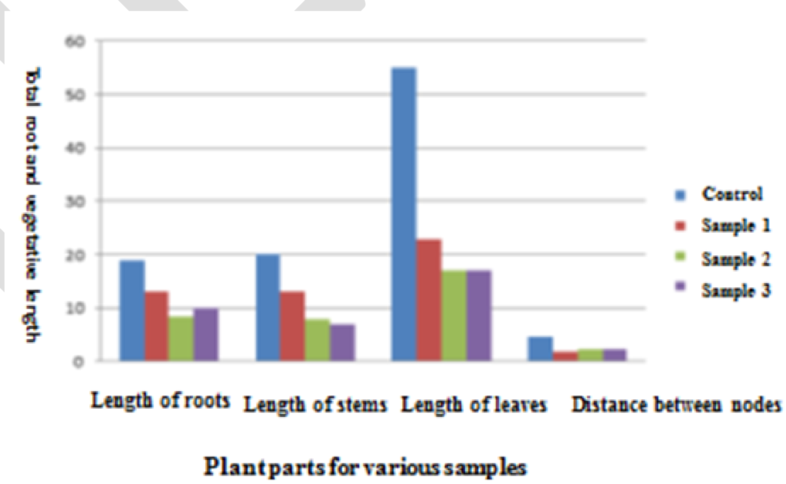

Figure4: Comparison of the length of roots and stems (cm) of corn plants inoculated with *F.roseum* by spraying the aerial parts with the control.

3.2- Treatment after infecting corn plants of *F.roseum* by spraying the overhead parts

Fourteen days after infection of *Fusarium*, all plants were treated with a spore suspension of *T. viride* (10^6 spores / ml), thus spraying all aerial parts.

After 22 days of treatment, several observations were recorded concerning the morphological characteristics of the plants (figure 5), it was noted the disappearance of the red color on the surface of the leaves and the stems of the plants, as well as the majority of seedlings took the green color after yellowing during infection (figure 6). The statistical analysis of the results at 95% level and the degree of freedom 2 refers to the calculated values 0,171- 0,233- 0,044- 0,270 for the parts of the plant tested (roots, stems, leaves, and the distance between the nodes) respectively, these values is greater than 0.05, Therefore, there is a difference between the average lengths of the tested plant samples, after being inoculated with *F.roseum*, and then treated with *T. viride*, this confirms the effect of *T. viride* on *F.roseum.* This results in the apparent regression of the symptoms of the disease and the increase in the length and size of the stems after they were thin. The length of the roots treated samples (1-2-3) reached (26, 5 – 31, 5 – 32, 5 cm) the length of the stems reached (20- 23, 5 – 23) cm the length of the leaves estimated from (55, 33 – 46, 36 – 53, 33) cm and finally the distance between the nodes was (2.42 - 3.35 - 3.21) cm respectively. In comparison with those of the control, in which the length of the roots was 34.5 cm, the stems with 23.5 cm, the leaves with 66 cm and the distance between the nodes estimated at 3.14 cm (table 2), (figure 7, 8).

Table 2: Length of roots and aerial parts of plants (cm) after 22 days of treatment of *T. viride* by spray

Plants parts / The samples	Total root length (cm)	Length of stems (cm)	Length of leaves (cm)	Distance between nodes (cm)
Control	34,5	23,5	66	3,14
Sample 1	26,5	20	55,33	2,42
Sample 2	31,5	23,5	46,36	3,35
Sample 3	32,5	23	53,33	3,21

Figure5: Comparison of the growth of control plants to those which treated with *T. viride* after 22 days of spray treatment of aerial parts of plants ((a) plants treated with *T. viride*, (b): control plants).

Figure6: Disappearance of characteristic symptoms of *F.roseum* after 22 days of treatment with *T. viride* ((a, b, c): widening of leaf surface, disappearance of spots and yellow color, disappearance of burns at limb margins, and leaves recovered the color green).

Figure7: Comparison of length of roots and aerial parts of control plants to those treated with *T. viride* after 22 days of spray treatment of aerial parts ((b): treated with *T. viride*, (a): control plants).

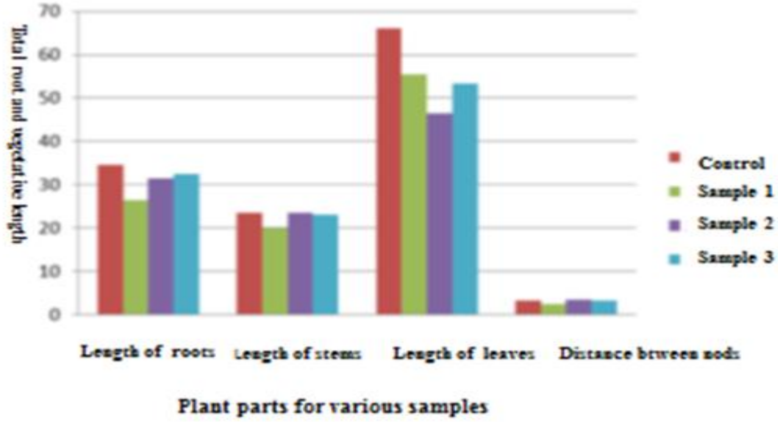

Figure8: Comparison of root length and aerial parts (cm) treated of *T. viride* untreated plants.

5- Discusion

Infection of the corn plant by a large number of fungi during their development can result in a loss of yield. *Fusarium* is probably infective to the roots, then develops and progresses densely, which stimulates their proliferation and causes the infection of neighboring plants. The pathogenic fungus continues to spread and infect from one year to the next, because of the germs found on plants or in the soil (Caron 2000).

Fusarium causes several diseases of the corn plant, including melting of seedlings, rot of stems and roots due to seed contamination. The disease is often observed in wetlands, and there is another source of fungi, the soil, where the fungus remains for a long time in the form of clamydospore. Seedling mildew disease is established in dry areas, which results in dryness in young seedlings in case of severe injury, and lateral roots fall in young stages (Gargouri, 2003).

In some cases, as the plant grows older, the fungus invades the stems, especially the young organs that constantly evolve and appear as white spots inside the marrow, often accompanying the dark red color on all parts of the plant. (Popescu, 2005) also points out those characteristic symptoms of the disease appear on the leaves during ripeness. During the interaction between the plant and *Trichoderma*, it contributes to the protection and enhancement of plant growth and soil fertilization. *Trichoderma* strains break down easily and also affect pathogenic fungi.

The ability of *Trichoderma* antagonism, is not only limited as a biological control agent, but it is considered a qualified agent for soil fertilization, preserves the integrity of the environment and helps to increase the fruit production and reduce the treatment of chemical compounds (Schirmbock and *al*., 1994)

Trichoderma is characterized by the production of large amounts of hydrolytic enzymes, which contribute to parasitism and secretion of enzymes (CWDE) capable of lysing the cell membrane of various hosts, including chitinase, 1,3-glucanase and proteases (Kubicek and *al*., 2001).

6- Conclusion

In the case of infection of *F.roseum* by spraying the aerial parts of the tested plants, after 14 days the manifestation of the characteristic disease symptoms of the inoculated fungus, consisting of leaf rolling and yellowing of the latter, was observed appearance of red color on the leaves of infected plants but less frequently, than it was when inoculating corn plants at ground level. There was a significant decrease in root length measurements, stems, leaves, and the distance between nodes compared with control.

After treatment of *T. viride*, there was a significant increase in measurements of different organs of the treated plants. Thanks to all these measurements and morphological characteristics, it can be said that the *T. viride* fungus plays an effective role in improving plant growth and also has the ability to resist fungal diseases associated with plants.

To demonstrate the effectiveness of *T.viride* against pathogenic fungi of plants in the applied field, we suggest future prospects:

- The study of the mechanical effect of *T.viride* on stopping the growth of pathogenic fungi.
- Determination of the tolerant range of pathogenic fungi and their susceptibility against *T. viride*.
- The use of *T. viride* in the field of agriculture to fight against fungal diseases
- Biotechnology study of *T. viride* leads to the extraction of their secondary metabolites, identifying these metabolites and then applying them in agriculture.

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