

Contribution to the study of *T. viride* activity on regression of symptoms caused by *F.roseum* and infestation of *Zea mays* seedlings

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Abstract

When inoculating corn plants with the *Fusarium roseum* spores with a rate of 105 spores/ ml at the soil level and after fourteen days of the infection, the symptoms of the disease appeared on most corn plants and a significant decline in the measurements of root length and vegetative parts compared with the control plants. The *Trichoderma viride* was tested against *F.roseum* where the infected corn plants were treated with *T.viride* with the average of 106 spores/ ml after twenty two days of the treatment, the symptoms disappeared and measurements of root length and vegetative parts approached the measurements of its counterparts. The stage of reisolation confirmed the existence of *F.roseum* on corn plants and the previously fertilized soil.

Keywords: *Zea mays*, test *in-vivo*, *Fusarium roseum*, *Trichoderma viride*, biological control

Introduction

Corn is among the plants that have a great importance due to the nutrients it contains. For the time being, it occupies the third position after wheat and rice (FAO, 2004). The reason for the spread of its cultivation in Africa lies in its ability to cope with the environmental conditions as it is also considered the most consumed product in many countries.

Corn plant is exposed to infection by many non-biotic factors (drought and pollution of the environment ect...) and biotic factors affect the plant development, which facilitates its infections by many fungal diseases and viruses (Ristanovic, 2001). These diseases cause losses in returns as in the quality of the nutritional and commercial value which consists of the inability to germinate, to analysis sugar, fats and proteins.

On the other hand, research works have continued to put as specific strategy which aims at deterring these pests or reducing its tension where there has been several ways contributing to fight plant diseases, including chemical resistance, which relies on the use of chemical pesticides. Resistance to these negative effects is reflected on the environment, human and animal health; and as a result harmful insects acquired the ability to resist these pesticides.

With the advance of research, an alternative method of chemical resistance has been discovered which consists of the biological resistance where ecological systems depending on monitoring pathogenic organisms through other organisms, including some insects arthropods, nematodes, bacteria and fungi (Lee and Lee, 2007).

The biological resistance is characterized by not leaving toxic on the environment and on human health, as well as not impacting other living harmful insect resistant organisms. It is also characterized by its usage easiness and no preciseness. Several studies showed the mechanism of the biological resistance of *Trichoderma* against pathogenic living microorganisms in soil, and which surrounds plant roots. These mechanisms consist the secretion of a set of extracellular enzymes and antibiotics (Bai and *al.*, 2008). This is what leads to a competition found in the external environment. This fungus is also characterized by its ability to incite plant resistance.

2-Materials and Methods

2.1- Studying the activity of *Trichoderma viride* fungus at the field level in- vitro

The study aims at confirming the ability of *T.viride* to minimize or control fungal diseases associated with corn plant after being tested in the laboratory.

2.1.1-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 50g 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 regularly watered by plain water twice a week with about 50ml for each pot and 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 5ml 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 mother liquid 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 10⁵ spore/ ml, and then the seedlings were inoculated (Gnancadja,2002).

2.2.2-Through soil inoculation on which corn seedlings were grown

When corn seedlings reached the stage of four to five leaves, each pot had been inoculated with an average of 50ml of sporal solution of *F.roseum* with a concentration of 10⁵ spores /ml (Nkongolo,1994).

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 10⁶ spore/ ml and kept in the refrigerator till use (Rojan, 2010).

2.2.4-Treatment of corn plants with *T.viride* after fourteen days of being infected by *F.roseum*

The study aims at testing the effectiveness of the *T.viride* against the impact of the pathogenic fungus on corn plants and thus fighting against the diseases caused by *F.roseum*. After fourteen days of the appearance of the diseases symptoms, corn plants were treated with a rate of 10⁶ spore/ml of *T.viride*.

2.2.5-Treatment of corn plants with spores of *T.viride* at soil level

The soil of the infected corn plants had vaccinated with an average of 50 ml per pot with the 10⁶ spores /ml of *T.viride* (Windham, 1986).

3-Results

3.1-Inoculating corn plants by the spores of *F.roseum*

This study aims at observing the development of the *F.roseum* disease on corn plants, and that is through the interventions between the pathogenic fungus and corn plants. It is attributing to the damage caused by insects before and after harvest. Added that, factors of the external environment like absolute humidity, during the production and before harvest, participate in the development as well as the soil sediments which contribute in helping in the creation of a physiological tension for the plants; which facilitates its infection with fungi. The *Fusarium* is among these living microorganisms associated with corn plant, which is considered the cause of many economic losses in most plants. This species siveeps over every vegetative and reproductive plant organ. On this basis, corn plants were inoculated by the *F.roseum* spores with an average of 105 spores/ml.

3.2-Inoculating corn plants by the *F.roseum* spores at the soil level

During the phase of five leaves of the majority of plants, the fungal spores of the *F.roseum* have been inoculated with a rate of 105 spores/ml at the soil level. After fourteen days of causing the infection, it has been noticed that there were symposium of the pathogenic fungus.

These latter have were demonstrated through a big decline in the plant mass and size. That is to say, an important lack in the plant length during the seedlings ascent stage, as the edges of most plant leaves dried out (figure 1). In addition, the deep red color was observed on the main vein of the leaf and covered the entire surface of the latter, the stems were colored in red, too (figure 2). The statistical analysis of the morphological features (characteristics) showed a clean difference between the totality of the infected plants with the pathogenic fungus as the control plants which are not infected. The *F.roseum* affected the growth of both roots and stems, which appeared to be very weak, along with a significant lack in the blade of the leaves; compared to the control plants that had a good size, greenness and the leaves surface breadth.



Figure 1: comparison of the growth of the control plants with the corn plants vaccinated with *F.roseum* after fourteen days of infection at the soil level (A: contaminated plants by *F.roseum*, B: control plants).



Figure2: symptoms of disease caused by *F.roseum* accompanying the various parts of corn plants after fourteen days of infection (A: appearance of red on the edges of the blade and the middle trunk, B: color of the stems with red color and the presence of a white pigment on this area , C: The red color covers all the leaf area).

It has also been recorded the absence of both dehydration and the reddish color in all the plants, beside the morphological observations on the infected plants. As a result, the length of the infected plants root groups (1-2-3) was evaluated to be (13,33- 12,33- 12,33) cm after fourteen days of growth, followed by stems length (12,4- 13- 13) cm (figure 3). The reason of the *F.roseum* infection, whose effect was very dear on the leaves which were characterized by a weakness in the growth and the surface blade small size, where its length was (28,66- 27,66- 23) cm. Finally, the distance between the nodes along the stems was estimated to be (1,84- 2,21- 2,29) cm respectively. The results obtained indicated a significant decline in the size of the three plants, which are statistically identical, however, with 18,33cm for the roots length, 18,5 cm for the stems, 55cm for the leaves and 3,92 cm for the distance between nodes in the control plants.

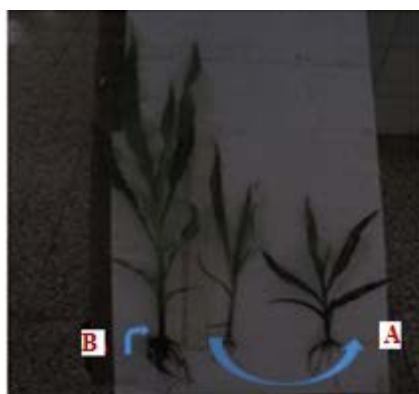


Figure 3: comparison of the total root and vegetation length of the control plants with those vaccinated with *F.roseum* after fourteen days at soil-level (A: Plants contaminated with *F.roseum* , B: plant of the control).

From all these measurements and morphological observations of all the infected corn seedlings, it is clean that infection at the level is quite serious (dangerous) and is expressed through a decline in the plant growth and development, where the plants stopped growing in the ascent stage (figure 4). The latter can cause the death of roots, for vegetative dryness and then death (table 1).

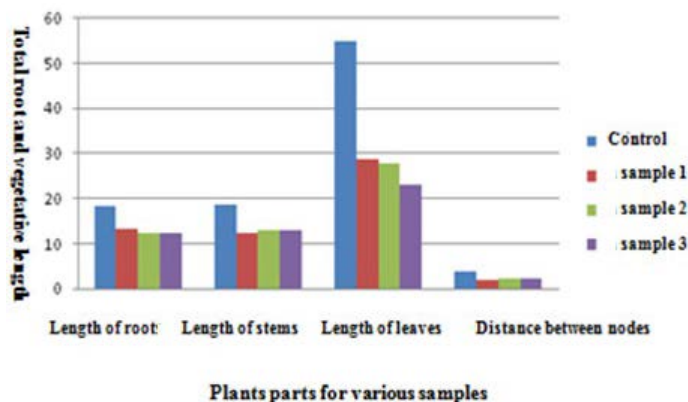


Figure 4: comparison between the total root and vegetative length (cm) of corn plants vaccinated with *F.roseum* at soil level and the control

Table1: total root and vegetative length (cm) of maize plants after fourteen days of soil vaccination with *F.roseum*

Distance between nodes (cm)	Length of (leaves (cm	Length of stems ((cm	Length of roots (cm)	Plants parts A sample
3,92	55	18,5	18,33	Control
1,84	28,66	12,4	13,33	sample 1
2,21	27,66	13	12,33	sample 2
2,29	23	13	12,33	sample 3

3.3-Testing the treatment with the *T.viride* after fourteen days of the infection with the pathogenic fungus

The choice of the *Trichoderma* species is quite important in the biological resistance domain, and it has no negative effect on the plants. A lot of species of the *Trichoderma* are characterized by a multi strategy in terms of contrast with the pathogenic fungus, and indirectly it has a positive effect on plants health that can be seen in enhancing and increasing its growth. Some other have the ability to produce antibiotics, where as another group (category) contribute mainly to food production. Generally, this species is characterized by some mechanisms that help in protecting the plants and fight fungi. The aim of this study is an attempt to treat the infected plants at the soil level and the seedlings infected by the *F.oseum* through spaying as follows.

3.3.1-Treating after fourteen days of the infection by the *F.roseum* at the level soil

The statistical analyses proved of the results of the preventive treatment with *T.viride* at a rate of (106 spores/ml) proved the effectiveness of this fungus in the resistance against the pathogenic one at the field level; and consequently, killing or curbing the fusariose disease, as the results recorded after twenty two days of the treatment show that the disease symptoms of the *F.roseum* regressed (fell back, withdrew) (figure 5, 6).



Figure 5: comparison of the growth of the control plants with the plants treated with *T. viride* after twenty two days of treatment at the soil level (A: plants treated w .(ith *T.viride*, B: control plants

After the appearance of the fusarirose disease, all the seedlings were treated by the sporale solution of the *T.viride* at a rate of (106 spores/ml). After twenty two days of the treatment, a lot of remarks concerning the morphological appearance of the roots and vegetative parts of the seedlings, and the measures of the plants parts were taken (figure 6).



Figure 6: the disappearance of the symptoms of disease caused with *F.roseum* after twenty two days of treatment of corn plants with *T.viride* (A: the breadth of the leaves, the disappearance of spots and the red color on the leaf area, B: increase the green color of the vegetative total.

The disease severity decreased and the pathogenic symptoms which covered all the parts colored in green had completely disappeared. It has been noticed, at the sometime, a total absence of the red color, as well as the dehydration that characterized a lot of leaves. In general , the majority of leaves recovered its vitality and increased their stem's length as the leaves expanded, the root system grew thick and the majority of the treated plants with *T.viride* didn't show (didn't demonstrate) any clear difference when compared with its control counterpart plants (figure 7).



Figure 7: comparison between the root and vegetative length of the control plants with those treated with *T.viride* after twenty two days at soil-level (A: plants treated with *T.viride*, B: control plant).

The total length of the sample roots (1-2-3) was estimated to (36,5- 37,5- 32) cm respectively. Regarding (with regards to, concerning, as far as the stem length is concerned, as far...). The stem's length, the leaves and the distance between nod was estimated to be (23-22-20) cm, (60, 59- 57,33) cm and (2,92- 2,5- 1,5) cm respectively. When making the comparison with the control plants, it has been found that the roots length were 39 cm, followed by stems 24 cm, the leaves 69,66 cm finally the distance between the nodes was 4cm (figure 8) (table 2).

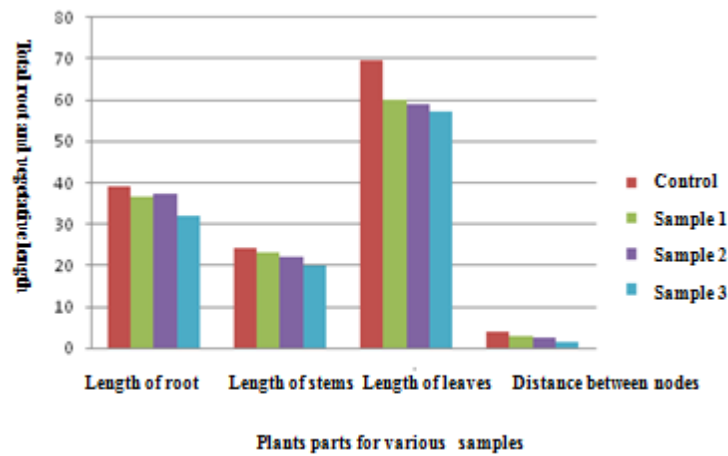


Figure 8: comparison between the root and vegetative length (cm) of the maize plants treated with *T.viride* . at the soil level and the control

Table 2: total root and vegetative length (cm) after twenty two days of treatment the maize plants with *T. viride* at soil level.

Distance between nodes (cm)	Length of (leaves (cm	Length of stems ((cm	Length of roots (cm)	Plants parts A sample
4	69,66	24	39	Control
2,92	60	23	36,5	sample 1
2,5	59	22	37,5	sample 2
1,5	57,33	20	32	sample 3

From the results obtained, it was clear that the infected plants with *F.roseum* (105 spores/ml), and after being heated with *T.viride* at a rate of (106 spores/ ml), it regained its vitality and activity and that was reflected in the convergence of roots length, of the treated plants and the stems with its control counterparts, with a small difference between the leaves and the distance between the nodes in the control and treated samples.

4-Discussion

Schisler and *al* (2002) clarified that the *Fusarium* causes infection to the plant and was known as the fundamental factor leading to wheat, barley and corn production loss. Among the widely spread on corn plants: *F.graminarum*, *F.moniliforme*, *F.oxysporium*, and *F.verticilloide*. Saunders and Kohn (2008) clarified that the fusarirose disease is translated into a lack in the ascent stage of plants. The first symptoms of the disease appear on the cleoptile which has dry spots then parasite dips into the primary rots and leaves. The research of (Yates and *al.*, 2005) confirmed that after vaccinating corn plants with *F.verticilloide* and other unvaccinated ones for the purpose of observing the plant growth

after the infection. It has been concluded that the *F.verticilloide* does not only cause a mass and size decline, but it may also exceed this to a decline in returns. Elena (2004) pointed out that corn plant catches many pathogenic parasites sweeping overall the plant parts starting from germination stage till maturity stage. Among these parasites, the *Fusarium* that causes considerable loss to the plant. This fungus appears on two consecutive stages: the vegetative phase of corn plant which starts from germination to the phase of three to four leaves and flourishing and then maturity phase. According to Gomes and al (1982); (Watson, 2007) corn plant gets infected during all the vegetative stage, starting from the roots that the fungus needs up to the leaves and that's in a short period of time. Then the latter falls down and dies. In some cases when the plant progresses in age, the fungus sweeps over the plant especially the young sweeps over the plant especially the young parts which grow constantly. It appears on form of white spots inside the pulp. These symptoms are often accompanied with dark red color on all the plant parts, the thing which its growth. These researchers also noted that the distinctive symptoms appear on the spikes during the maturity stage. *F..roseum* is considered somewhat a complicated fungus. It includes several basic types: *F.roseum* var, *culmorum*, *F.roseum* var, *graminearum*, *avenaceum*, *F.roseum* var, *arthrosporide*, *F.roseum* var. This species were noticed on plants like wheat, barley especially corn during the vegetative phase of the ascent stage of the plant till harvest. Among the symptoms with which *F.roseum* is characterized during the infection of corn plant, drought of leaves, the emergence of black color between nodes while the stem gets a red dye (Champion,1997).

5-Conclusion

The fungus penetrates plant tissue through natural openings (gaps, skin cells) or wounds on followed by the vaccination of corn plants stage five leaves at the level of soil with fungus *F.roseum* rate of 105spore / ml, to determine the pathogenic symptoms of this fungus. After fourteen days of infection, symptoms of the disease appeared on corn plants, which are a significant decline in the size of plants, a decrease in the length of the latter during the rise, the appearance of dark red on the leaves and stems. Also record declines in roots length measurements, stems, leaves and the distance between the nodes of samples (1 -2-3) tested and compare them with the control. To evaluate the efficacy of *T.viride* against certain diseases caused by fungal strains affecting maize crops, including the *F.roseum* mushroom, whose effect is on the morphological and physiological characteristics of the maize plant? After treatment of maize plants with *T. viride* with a rate of 106 spores / ml after twenty two days of treatment, there was a significant reduction in the symptoms of the disease and the disappearance of the red color, dryness of leaves, increased stems length and the width of the limb surface of most treated plants. According to several studies that show the ability of this fungus to produce a huge amount of metabolic products, whether enzymes, antibiotics...ect. Based on the above results, we propose, as a future prospect, the extraction of the organic products of this fungus, its definition and the introduction of some amendments for its application in the field of agriculture.

6-References

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