COMPARATIVE STUDY BETWEEN THE EFFECT OF TRICHODERMA VIRIDE AND DIFENOCONAZOLE TO INHIBIT GROWTH MYCELIAL Of FUSARIUM OXYSPORUM

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Abstract

Direct confrontation tests between T. viride and F. oxysporum on agar medium (PDA) revealed considerable activity by the biocontrol agent on the pathogen. After six days of incubation, the average diameter of the pathogen colony was 26 mm and the percent of inhibition was 63.83% relative to the control with 71 mm. At the end of the eighth day, T. viride completely invades the F. oxysporum colony and sporulates on it.The results of the test fungicide revealedmoderate inhibition of mycelial growth of the pathogen, so the concentration of 300 ppm was considered a threshold of inhibition of mycelial growth of F. oxysporum, which appeared sensitive.Otherwise, F.oxysporum appeared resistant against the different concentrations (30 and 3) ppm of Difenoconazole.According to literature and several studies carried out in this field, it has been concluded that T. viride is very effective against pathogenicity of F.oxysporum, whereas the use of fungicide (Difenoconazole) is effective but with varying degrees.

Keywords **:** T.viride, F.oxysporum, Difenoconazole, activity, comparative.

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INTRODUCTION

Fungicides are products that kill or inhibit the fungi that cause certain diseases. Such as fungal diseases that cause severe damage to cultivated plants [1].With the pesticides, human have been able to control their food crops, escaping the spread of many pests. Fungicides based on copper Sulphate are spreading, especially the famous bordeaux mixture (a mixture of copper sulphate and lime) to fight fungal outbreaks of potatoes. Mercury salts are used at the beginning of the 20th century for the treatment of seeds. The Benzimidazole and Pyrimide fungicides date back to 1966, followed by the Imidazole and Triazolic fungicides known as the Sterol synthesis inhibitor (IBS) fungicide [2] .

After the adverse effects of the use of chemicals on the environment (appearance of resistant strains and accumulation of fungicide residues), control of plant infections caused by fungal pathogens is also considered more frequently by a biological approach [3]. Interest in biological control has greatly increased in recent years.

*Trichoderma*species have received considerable attention as a biological control agent against a number of soil-borne pathogens. Research on mechanisms to control pathogenic populations in the rhizosphere suggests that the antagonistic activity of *Trichodermasp* lies in the production of extracellular enzymes and / or antibiotic substances [4].

The aime of study is to determine the inhibitory capacity of *T.viridein -vitro* against a telluric fungi*F.oxysporum*. We therefore found that this agent plays a very important role in the inhibition of the development of the pathogen, either in direct contact or in distance, which will allow the future to use it *in- vivo*, then to suppress the proliferation of pathogens.

MATERIAL AND METHODS

1. Biological material

1.1. The antagonist agent

The biocontrol fungus used in this study is *T. viride*, it has been isolated from the soil of the Jijel region where it has grown the maize plant.

The identification of the biological control agent was carried out based on morphological characters (macroscopic and microscopic study), the identification is carried out in the Laboratory of Mycology, Biotechnology and Microbial Activity, Department of Microbiology and Biochemistry, University Mentouri Constantine1 Algeria.

1.2. The pathogen agent

The isolate of *Fusariumoxysporum*used in this study was obtained from palm leaves grown in Oumech commune (Biskra).Algeria. The pathogen is identified in laboratory of microbiology. Department of Environmental Sciences and Agronomic Sciences. University Jijel.

1.3. The fungicide

The fungicide used is Dividend®, it applies on seed and the grains, used against pests, charcoal, square and *Septoria*, it is in soluble form (Table1).

Table1: Fungicide information

Trade Name	Active ingredient	Approved dose	Used
Dividend®	Difenoconazole 30g / l	20 ml / KMTL seed	Treatment of seeds against pests, charcoal, *Fusarium*, *Septoria*

METHODOLOGY

1. Antagonist activity of *Trichodermaviride* against *Fusariumoxysporum*

The confrontation test between *T. viride* and the *F.oxysporum* strain was carried out in Petri dishes containing the medium (PDA), two mycelial disks 5 mm in diametre (the first containing *T. viride* and the other carrying *F.oxysporum*) are placed diametrically on the culture medium.

Incubation is carried out at 25 ° C for six days. Notations concerning the inhibition of diametral growth of *Fusariumoxysporum* colonies and their invasion by *T. viride*mycelial were performed daily. The control contains only the pathogen (*Fusariumoxysporum*) in the center of the Petri dish containing the medium (PDA).

The evaluation of the inhibition exerted by the antagonist agent (*T.viride*) is estimated by calculating the percentage inhibition of mycelial growth according to the following formula [5; [6].

I% = (1-Cn/Co)× 100

Or:

I (%): percentage inhibition of mycelial growth.

Cn: the average diameter of the colonies in the presence of the antagonist.

Co: the average diameter of control colonies.

2. Fungicidal-pathogenic test

The choice of concentrations is making on the basis of preliminary tests and the work of some authors [7] ;[8] .

3. Fungicide test –*Fusariumoxysporum*

The test is performed according to the method [7]. Fungicides suspended in sterile distilled water are diluted to the desired concentrations (3000, 300, 30, 3) ppm. Sterile watman paper disks with 5 mm of diametre are impregnated with 1 ml for each concentration and previously deposited on the PDA agar in Petri dishes. The distance between the pathogen and the soaked discs with fungicide is well defined. The dishes are incubated at a temperature of 25°C for six days. The control consists only of a disk containing the pathogen in the absence of disks soaked with fungicide (Fig 1).

With:

1- Sterile wattman disk soaked with fungicide.

2- Disk carrying *F.oxysporum*.

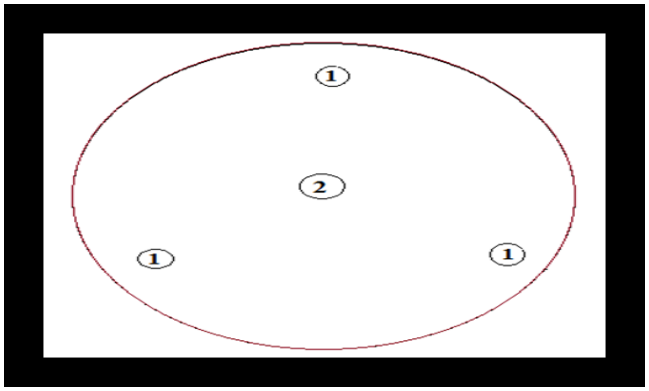

Fig 1. Antagonism test between *F.oysporum* and Difenoconazole on PDA medium at 25°C

RESULTS AND DISCUSSION

The purpose of this study is to test the ability of the antagonist to suppress the growth of the tested pathogen. So, the direct confrontation test shows an inhibitory effect of *Trichodermaviride*against *Fusariumoxysporum*isolate.

1. Direct confrontation on PDA medium between*Trichodermaviride*and *Fusariumoxysporum*

The study of mycelial growth of colony of *Fusariumoxysporum* confronted with *Thrichodermaviride*, shows a significant reduction of the growth of the pathogen compared to the control (Fig2).Simultaneous transplantation of *T.viride* and *F.oxysporum* showed a faster growth of *T. viride* than that of *F. oxysporium* isolate. After 3 days of incubation, *T. viride* invades almost the entire surface of the dish, whereas *F.oxysporum* occupies only 23.5 mm in diametre, and the growth of *F.oxysporum* is stopped during the fourth days with a rate of low growth, which corresponds to the percentage inhibition of mycelial growth with 63.83% (Fig3) (Table 2).According to [5], this interpretation is due to the action of the enzymes (β1-3) gluconase-chitinase which leads to lysis of parasite mycelial.

Table 2: The average diameter of colonies (mm) of *F. oxysporum* in the presence of *T.viride* compared to the control

Colonies / Days	Average diameter of *F.oxysporum* colony	Average diameter of control	Percentage of inhibition %
1	13.5	15.5	12.90
2	21	24	12.5
3	23.5	39	39.74
4	26	55	52.72
5	26	65	60
6	26	71	63.83

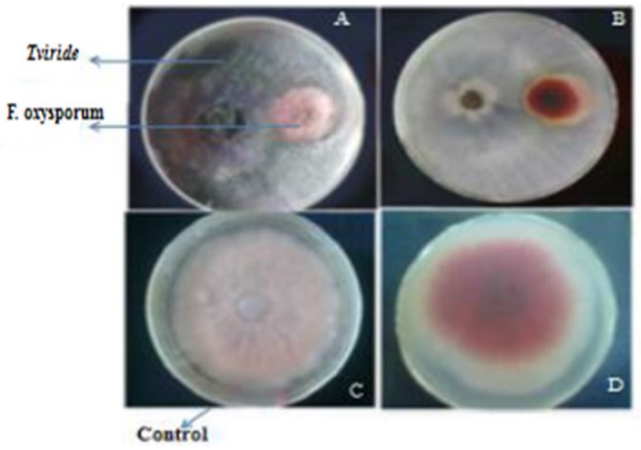

Fig 2. Inhibitory effect of mycelial growth of *F. oxysporum* in the presence of *T. viride* after 72h of incubation at 25 ° C (A (recto): confrontation test, B (verso); C (recto): control colony; D (verso)

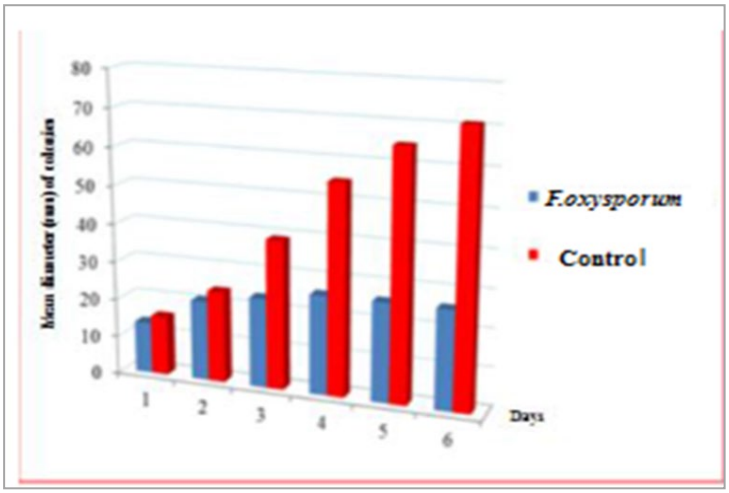

Fig 3. Comparison between the mycelial development of *F.oxysporum* in the presence of *T.viride* and the untreated control

In addition, *Trichoderma* is a fungi that naturally colonises plant soils and roots before phytopathogens, and may play a predominant role in plant health [9]. Various researchers have reported the antagonistic activity of different Trichoderma isolates against phytopathogenic fungi such as *R. solani*, *F. oxysporum* and *Sclerotiumrolfsii* [10].

Control grown alone occupies a surface of 71 mm in diameter after 6 days of incubation; with a light pink aerial mycelial in the early days of its development; then takes a pink-vermilion colour.

After ten days of incubation, it was found that *T.viride*sporulates on the *F.oxysporum* colony, and appears granulose on the surface of the pathogen colony (Fig4).The same results reveled by Otherwise, [11] found that the strain of *T.viride* has an important activity of secreting enzymes in order to attack or suppress the mycotoxinssynthesised by phytopathogens. After six days *T.viride* completely covers the colonies of pathogens on which it sporulates, *T.viride* showed an antagonistic power, which is the ability to stop or suppress the development of pathogens *Verticilliumsp* and *Phoma sp4* [12].[13] observed the invasion of the pathogen colony by *Trichodermaharzianum*, *in- vitro* between this antagonist and *Sclerotinasclerotinium*; [14] conducted a direct confrontation between *Trichodermaharzianum* and a telluric fungus, *F.oxysporum* on medium (PDA).

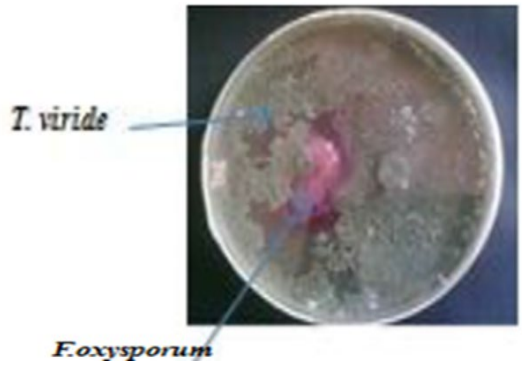

Fig 4. Sporulation of *T.viride* on the *F.oxysporum* after ten days of incubation at 25ºC on PDA medium

2. Fungicide test- *Fusariumoxysporum*

The reduction of mycelial growth is low to medium for the different concentrations of the fungicide (Fig5). The inhibition percentages vary between (16.40 and 64.95) %. After four days of incubation, the mycelial growth of *F. oxysporum* was stopped after treatment with 3000 ppm and 300 ppm, and reached an average diametre of 18.5 mm and 27 mm respectively, whereas, inhibition of mycelial growth of *F. oxysporum* reached 42 mm and 51 mm after five days of incubation, and after treatment with 30 ppm and 3 ppm respectively (Fig6).The concentration required to inhibit 50% of *F.oxysporum*mycelial growth is the 300 ppm of the fungicide used (Table 3).The control of *F.oxysporium* grown alone occupies a surface of 79 mm in diametre after six days of incubation[15]. In addition, [16] showed that systemic fungicides such as Triazoles and Difenoconazole are effective against several cereal diseases. The use of pesticides helps prevent the spread of certain diseases, such as the mildew, transmitted by parasitic fungi.

Total inhibition was observed for the isolate tested at 3000 ppm Difenoconazole. There are also variable responses of the isolate to other concentrations. Where 50% inhibition of mycelial growth of *F.oxysporum* is recorded at 300 ppm compared to the control. This raises the problem of resistance to fungicides and the proliferation of the pathogen in a normal way. Fungicides are one of the means of fight against phytopathogenic fungi and must contribute.Pesticides have a negative impact on ecosystems (water pollution, poisoning of bees, birds or earthworms, etc.). Many of these pesticides are toxic to living organisms. A chemical that kills a fly can also kill a dog. The repetitive use of the same product and sometimes misguided pesticides gives rise to insects, plant diseases, weeds resistant to some of these products [17].

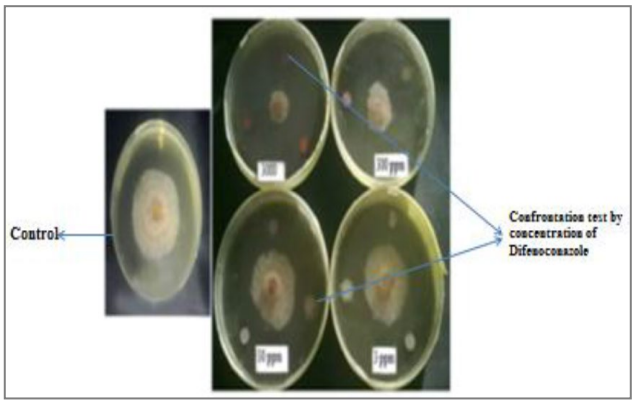

Fig 5.Effect of different concentrations of the fungicide on the mycelial growth of *Fusariumoxysporum* after four days of incubation at 25ºC

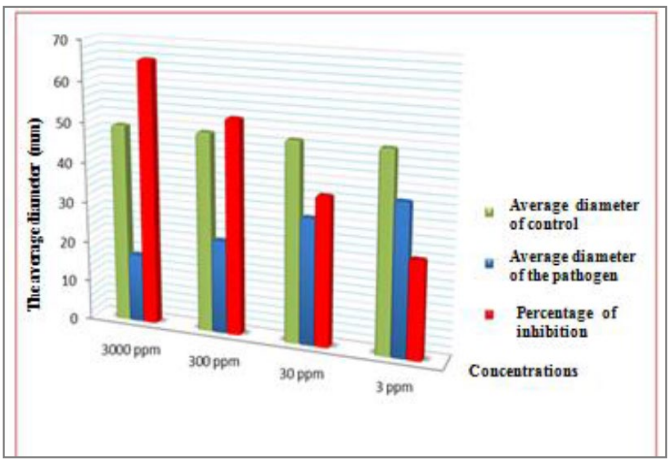

Fig 6. Average diameter of colonies (mm) of *F.oxysporum* treated with the fungicide (deferent concentrations) and inhibition rate after six days of incubation

Table 3:Average diameter of colonies (mm) of *F. oxysporum* treated with the fungicide (different concentrations) and inhibition rate after six days of incubation

Difénoconazole (ppm)	First day	Second days	Third days	Fourth days	Fifth days	Sixth days	Average diameter (mm) of colonies	Percentage of inhibition
3000	13.5	15	17.5	18.5	18.5	18.5	16.91	64.95
Control1	14	23	45	61	69	77.5	48.25	
300	14	20	23.5	27	27	27	23.08	52.65
Control 2	14.5	23	44	63	70	78	48.75	
30	14	23	31	41	42	42	31.16	35.19
Control 3	14.5	25	42	61	69	77	48.08	
3	14	24.5	35	49	51	51	37.41	16.40
Control 4	14	24	43.5	64	71	79	44.75	

Pesticides protect plants or plant products against all pests or prevent their action, and destroyed the undesirables. In addition, many pesticides are toxic, therefore dangerous for human health (congenital malformations, cancers, neurological disorders, fertility and immune system).

CONCLUSION

This work has two parts: biological control and chemical control. The first part is devoted to the study of the effect of biocontrol fungi using *T.viride* against *F.oxysporum*.The effect of *T.viride* is manifested by an inhibitory activity of mycelial growth of *F.oxysporum* with a percentage inhibition that reaches 63.83%.The second part consists of an *in- vitro* test using Difenoconazole (chemical control) against the *F.oxysporum* isolate. It has been shown that the 300 ppm concentration shows a 50% reduction in mycelial growth, so the tested isolate is more susceptible to fungicide.

According to this study, *T. viride* has been shown to play a very important role in biological control, while the use of Difenoconazole fungicide with respect to *F. oxysporum* shows a variability of inhibitory action according to the concentration (300 ppm).

PERSPECTIVE

The realisation of this study has enriched our knowledge of biological and chemical control; and we can afford to fix these points as perspectives.

- The application of *in- vitro* tests perform by *in- vivo* tests.

- The widening of the range of the studied species in order to deepen the results and to understand the interactions between the species.

-Study the side effects of chemical control on the environment and human health.

-The application of *in- vivo* tests in natural conditions.

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