Efficacy Of Using *Trichoderma spp* On Pathogens In Maize To Control Root Rot Disease

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Abstract

In the current study, biotic (*T.harzianum, T. konignii*, and *B. subtilis*) were used to examine the antifungal activity of these agents against maize root rot pathogens (*F. oxysporum, F. solani* or *R. solani) in vitro*. For these reasons, the experiments were done in the laboratory. Confrontation among biotic agents and the tested pathogenic fungi results shows that both *Trichoderma spp* and *Bacillus subtilis* prevent the linear growth of all the examined pathogenic fungi. *T.harzianum* appeared high level and significant effect of antagonism and diminuend the growth of *F. oxysporum*. While *T. konignii* was stronger in decreasing the linear growth of *F. solani* and *R. solani*. The antagonistic bacteria came an end as compared with antagonistic fungi in decreasing the linear growth of all examined pathogenic fungi.

In screening of the bio-agent antagonist test, it was observed that the negative effect was estimated by using *T. harzianum* on IZ, L against *F. oxysporum*, and on OG as well as L on *R. solani*. While it gives of a potential antagonism on the three measures against *F. solani* as well as OG when grows rapidly over the mycelium of *F. oxysporum* and occurs IZ on *R. solani*. Moreover, *T. koningii* grows considerably faster than three tested pathogenic fungi and occurs lysis in hypha of both *Fusarium spp* viz, *F. oxysporum*, and *F. solani*. On the other side, the antagonistic *B. subtilis* lead to presence of an inhibition zone in all tested.

Keywords: pathogens- bio agents - Root rot-*Trichoderma spp* and Maize.

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Introduction

Maize are infected by many pathogenic fungal diseases worldwide, which greatly limit crop production. *Fusarium spp*. It has shown the most severe diseases affecting many commercially domesticated grassy crops. A chemical fungicide to control it seems important because seeds and soil that help the transmission of the disease. Potent antifungal formation of bioagents including antifungal microorganisms has been reported to be useful for the biocontrol of these fungi in pulses under field conditions. However, the effect of biological control affects microbial and plant interactions and the ecological fitness of the bioagents.

Abdel-Razek *et al.*, (2012) It was found that the symptoms of root rot are dwarfism. It was found that the symptoms are rot in the roots. change in the color of the plants, short stem size, and the plants become weak and easy to infect more than disease-

resistant plants, and in many cases, the roots disappear. Delegation the cause of root rot disease was determined by the *Rhizoctonia* fungus. Root growth and development are weak. It includes obvious symptoms of root rot and its color to brown**.**

Mackenzie and Williams, (2015) found that yield losses due to fungal diseases have been estimated depending on the environmental conditions surrounding the plant. Yield losses are due to the presence of different pathogens up to 60%.

Da Silva, *et.al* (2017) found that maize (*Zea mays* L) is a crop of most significant cereals in the world. Both biotic and abiotic factors have an impact on the yield of this crop, as phytopathogenic fungi play a crucial role by lower yields levels and causing massive international economic damage. The phytopathogenic fungus *Rhizoctonia solani* is a significant maize pest that attacks all underground parts of the plant, including seeds and roots. In maize, *R. solani* is the causative agent of leaf in addition to sheath blight, although there are other symptoms such as leaf and sheath blight.

Rashad and Moussa, (2020) found that pathogens cause huge losses in agricultural production and cause huge losses in many countries, and that necessary measures must be taken to maintain manufacturing to supply a growing population needs. The pathogens led to a significant decrease in the crop, therefore, the injury leads to many economic losses.

Nohra *et al.*, (2021) Legumes are infected by many pathogenic fungal diseases worldwide, which greatly limit crop production. *Fusarium spp.* It has shown the most severe diseases affecting many commercially domesticated legume crops. A chemical fungicide to control it seems important because seeds and soil that help the transmission of the disease. Potent antifungal formation of bioagents including antifungal microorganisms has been reported to be useful for the biocontrol of these fungi in pulses under field conditions. However, the effect of biological control affects microbial and plant interactions and the ecological fitness of the bioagents. Hence, the present evaluation aims to provide summary data on *Fusarium* pathogenesis, fungal-host pathogen interactions, and biological control strategies in relation to legume crops.

Sivan and Chit, (1986) concluded that the application of antibiotic agents proved superior in monitoring a diverse range of plant diseases in several countries. At the same time, however, it can act as a better control measure *in vivo*. Recently, increasing worry about the utilization of pesticides on human health in addition to the environment has led to rising advantage in use options available. It has unfavorable effect on the environment. Therefore, we must focus on safe pesticides for humans and the environment. As an alternative to fungicides, a strategy for boosting plant defenses that offer defense against a variety of pathogenic organisms is possible among the artificial inducers.

Biological control of soil-borne pathogens is frequently credited to greater feeding that enhances host defenses or to direct suppression of pathogen growth in addition to action. Modification with a specific biological agent (inducers) seems to increase

disease resistance by indirectly boosting native populations of microorganisms that are good for plant growth in addition to anti-pathogens.

Trichoderma viride and *Trichoderma harzianum* were found by **Ghaffar, (1993)** to prevent the growth of all root rot fungi. Soil infection with any one of the tested biocontrol agents decreased the percentage of infection of plants with diseases.

T. harzianum stimulates plant resistance to soil-borne pathogens, while *B. subtilis* forms secondary metabolites that inhibit pathogens. To effectively combat soil-borne diseases, a variety of tactics are typically needed improved performance, yield management in addition to biological control agents. *Trichoderma* species are excellent competition for infection, can amend root coats, are tolerant or resistant to pesticides, can grow and endure in unfavorable circumstances, are efficient in using soil nutrients, have complete aggressiveness against phytopathogenic fungi, and promote **Asaka & Shoda, (1996).**

Jensen *et al.*, (2001) conducted a correlation between seeds infected or contaminated with pathogens. It was found that treating seeds with a small dose of fungicides or biocides improves the rate of seed emergence and reduces the disease percentage.

Abu Zaid *et al., (*2003) reported that the application of biological resistance may not be beneficial in all environments due to the different soil properties. Accordingly, it was found that the application of the biological control program is better in the resistance of pathogens and expands the scope of biological control activity, and enhances its efficiency of it. Therefore, in developing countries, the formulation of biological control agents is indicative of strong disease management, where the costs of chemical treatments may be very expensive. Many of the isolates of *Trichodrma spp*. Fungal pathogenic fungi could be, as a result of restoring the beneficial balance of the natural ecosystem, The result showed that treating the seeds with *Trichoderma harzianum* caused a decrease in the symptoms of wilting *Fusarium spp*. Induce damping -off also *R. solani* in faba bean, lentils, and chickpeas.

Abbas *et al.*, (2019) indicated that the biological control of plant pathogens is an substitution option to the utilization of fungicides. As the use of bio-agents, which can be used to eliminate pathogens. Bio-agents prevent the growth of plant pathogens by releasing antibiotics, inducing systemic resistance, in addition to competing for resources in the host plant and in roots.

Noor, (2020) confirmed that *Trichoderma spp*. It has been commonly applied as a biological control agent (BCAs). In the majority of agricultural operations. The use of this BCAs vaccine as *Trichoderma* has produced many attention-grabbing antimicrobial products to determine the significant superiority of *Trichoderma spp*. Which is characterized by the presence of many mechanisms such as combating plant diseases, stimulating plant growth, and. More than that, their secondary metabolites are generated in the agroecosystem. These enormous advantages allow *Trichoderma* to be used in agricultural production for the application of environmentally safe farming practices.

Hence, the present evaluation aims to provide summary data on biological control strategies in relation to grassy crops.

The aim of this study:

This study aimed to avoid the use of fungicides and reduce infection by biological control and natural stimuli in maize plants and to fight these pathogens using antibiotic organisms.

Materials and Methods.

Isolation and identification of the pathogens:

Samples of maize plants bearing clear signs of damping–off, root rot, and wilt were combined from dissimilar locations in Al Baha province, Saudi Arabia. The roots that were infected with diseases were prepared by washing them with tap water, then dividing them into small parts (about 1 cm). Thus, the diseased roots are prepared. Infected roots were divided into small pieces and then placed in a 0.5% sodium hypochlorite solution for two minutes, then put in sterile water for one minute and between two filter papers for drying. The prepared plant roots were placed in Petri dishes containing medium (PDA) with Add the antibiotic streptomycin 0.01% to prevent bacterial contamination. The dishes were positioned in the incubator at 25degrees Celsius and preserved at 4°C in a refrigerator for subsequent studies. After five days, each isolate was purified by isolating the tip of the mycelium after colonies were removed from the fungal mycelium **Sinclair and Dhingra, (1985).**

Purification of pathogenic fungi:

Fungi are purified by taking a part of the fungal mycelium and growing it on was a pure PDA environment. The resultant growth of the isolated fungi was identified depending on their morphological characteristics with light microscope description of **Gilman (1957); Booth, (1985),** and **Barnett and Hunter, (1986).** He made an estimate of each type of pathogenic fungi that was isolated and calculated the frequencies for each of them.

Pathogenicity tests:

Pathogenicity tests were performed *in vivo* AI-Baha – Shatiba 2022 Four isolates of *F.oxysporum* , three isolates of *F.solani*, and five isolates of *R.solani*. These isolates were tested to select fungi that have a high ability to cause infection. These seedlings (25 cm) were disinfected with 5% formalin for 15 minutes before being utilized in culture and then left in the open air until dryness. The inocula of the isolated fungi were prepared on sand-barley (SB)medium (25g clean sand,75g barley, and sufficient water to encompass the mixture) the additive mixed and bottled then autoclaved for 20 minutes at 1.5 air pressure, flasks containing sterilized medium were inoculated with agar discs acquired from the periphery of each isolated colony of 5-day-old fungi It was incubated at 25 °C for two weeks. By thoroughly mixing the soil with a 5% formalin solution, the soil was sterilized. Then the treated soil was coated plastic sheet for a week and then the plastic sheet was taken away to permit for this complete evaporation of formalin **Whitenhead, (1957)** soil infestation with each individual fungal was carried out at the rate of 3% of soil weight **Metwelly,(2004)** soil mixed thoroughly with fungal inoculum then watered daily for one a

week to boost fungal growth, the soil of monitoring pots were blended with the same amount of sterilized fungus-free sand- barley (SB)medium, Apparently healthy of maize seeds were surface sterilized using 2% sodium hypochlorite for 2 min, washed varied times with sterilized water than sow at an average of 10 seeds per pot, Three replicate pots with a total of 30 seeds were used for each treatment disease assessment:

The deadening percentages per and post emergence above the soil surface were determined as well as the percentage of surviving plants 15, 30, and 45 days after sowing, respectively, by the equation based on **El-Helaly *et al.*, (1970).**

Per-emergence (%) =Number of non-germinated seeds "at15days" / a Total number of sown seeds ×100.

Post-emergence (%) = Number of dead seedlings "at 30 days" / a Total number of sown seeds ×100.

Survived plant (%) =Number of survived plants "at 45 days" / a Total number of sown seeds ×100.

Bio agents isolation and identification of:

The biocontrol agents *Trichoderma harzianumm, Trichoderm koningii*, and *Bacillus subtilis* were previously isolated from the roots of healthy maize plants at the same site and used to isolate disease-causing pathogens. Twenty-five grams of soil sample was unsettled in 250 ml of 0.1% water agar and then shaken for 30 min on a rotary **shaker *et al.*, (2007)** at 250 ppm. Serial dilutions of each soil sample were performed, and 0.1 ml aliquots of soil suspension were dispensed into PDA medium for fungal bio factors and nutrient agar medium for bacterial bio factors in Petri dishes, and three dishes were considered replicas for each soil sample. The plates were incubated at 25°C for seven days. The developing fungus was purified or recognized according to **Bissette, (1991)**. while developing bacteria move to a selective medium according to **Ronald, (1993)** and identified according to **Bergey *et al.*, (2009).**

Evaluation of bioagents on linear radial growth of pathogenic fungi:

In-vitro inhibitory activity of bioagents against maize root pathogenic fungi was examined in dual culture method by a technique by **Kucuk and Kivanc, (2003)** Petri dishes containing sterile PDA were inoculated with a 5 mm length disc of a 5-day culture of antagonistic bioagent *Trichoderma spp* and pathogens. Fungi, pathogens, or Bio-agents were placed in Petri dishes by placing a disk of each of them in the Petri dishes on the opposite side of the other in a PDA environment. The dishes were incubated at 25°C. After that, the growth of the pathogenic fungus was measured. A dish containing only the pathogenic fungus was used. Control. It was repeated for each treatment at a rate of 3 replicates. *B. subtilis* inhibition of mycelial linear growth of both *F. oxysporum, F.solani, and R. solani* were determined. The aggressive bacteria isolates were grown on nutrient agar for 50 hours at 29 °c. the inhibition effects of antagonistic bacteria on the fungal pathogens were done by sprinting the antagonistic bacteria on side of the Petri dishes surrounding one disc of the fungal root pathogens on the PDA medium surface.

karan *et al.*, (1997). Check treatment was done by growing one disc of pathogenic fungus in the same place of treatment free of antagonistic bacteria and incubated for seven days at 28^{O}C in dark. average mycelial linear growth was measured when mycelial growth covered the surface plate in the control treatment. Fungal growth inhibition was calculated for each fungus tested for the growth control recommended by **Dennis and Webster, (1971).**

Screening of bioagents antagonist, *in vitro*:

According to **Melo and Faull, (2000),** The reaction of biotic agents against pathogenic fungi was estimated as Inhibition Zone (IZ), Overgrowth (OG) and Lysis (L) as shown in the laboratory tested .

Results:-

Pathogenicity tests:

testing for pathogenicity *F. oxysporum* isolates four, *F. solani* isolates three, and *R. isolates* five. For their ability to be the pathogenic agents causing pre- and post-emergence damping-off in maize plants. Information in Table (2) showed that the most examined isolated fungi were pathogenic then causes typical signs of both pre and post-emergence damping-off with different degrees. *R. solani* No1 was the most aggressive isolate on the base of seedlings mortality recorded at 60% survival plants. *F.oxyporum*(3) came next followed by *F.solani*(3) giving 63.33% and 70.0% survival plants, respectively. It is worthy to mention that *F.oxyporum*(1) had no effect on pre-emergence damping-off. Moreover, the same trend occurred under *F.oxyporum*(4) and *F.solani*(2) in post-emergence damping-off. Hence, the highest percentages of survival plants (96.67%) was recorded under both infection of *F.oxyporum*(1) and *F.oxyporum*(4) then *F.solani*(2) which gives (93.33%). Based on these results *R. solani*(1), *F.oxyporum*(3), *and F.solani*(3) were selected for further investigations.

Table (2): Pathogenicity test of the fungi isolates from rot root maize *(Zea mays* L*)*

	Damping off %		
Isolated fungi	***pre%**	****post%**	*****sur%**
F.oxyporum(1)	**0.00 d**	**3.33 cd**	**96.67 a**
F.oxyporum (2)	**3.33 cd**	**3.33 cd**	**93.34 ab**
F.oxyporum(3)	**20.00 a**	**16.67 ab**	**63.33 de**
F.oxyporum(4)	**3.33 cd**	**0.00 d**	**96.67 a**
F.solani(1)	**6.67 c**	**3.33 cd**	**90.00 abc**
F.solani(2)	**6.67 c**	**0.00 d**	**93.33 ab**
F.solani(3)	**16.67 a**	**13.33 b**	**70.00 d**
R. solani (1)	**20.00 a**	**20.0 a**	**60.00 e**

R. solani (2)	**6.67 c**	**6.67 c**	**86.66 bc**
R. solani (3)	**3.33 cd**	**6.67 c**	**90.00 abc**
R. solani (4)	**10.00 b**	**6.67 c**	**83.33 c**
R.solani (5)	**3.33 cd**	**3.33 cd**	**93.34 ab**

There is no significantly difference between figures in the same column that begin with the same letter(s). ($p \leq 0.05$).

***pre% = pre-emergence damping off **post% =post-emergence damping off and ***sur% =survival plant.**

Biotic-agents vis pathogens linear growth:

Information in Table (1) shows the impact of bio-agents i.e. *T.harzianum, T. konignii*, and *B. subtilis* on the mycelia linear growth of the tested pathogenic fungi. Both *Trichoderma spp* and *Bacillus subtilis* suppressed the growth of all the examined pathogenic fungi.

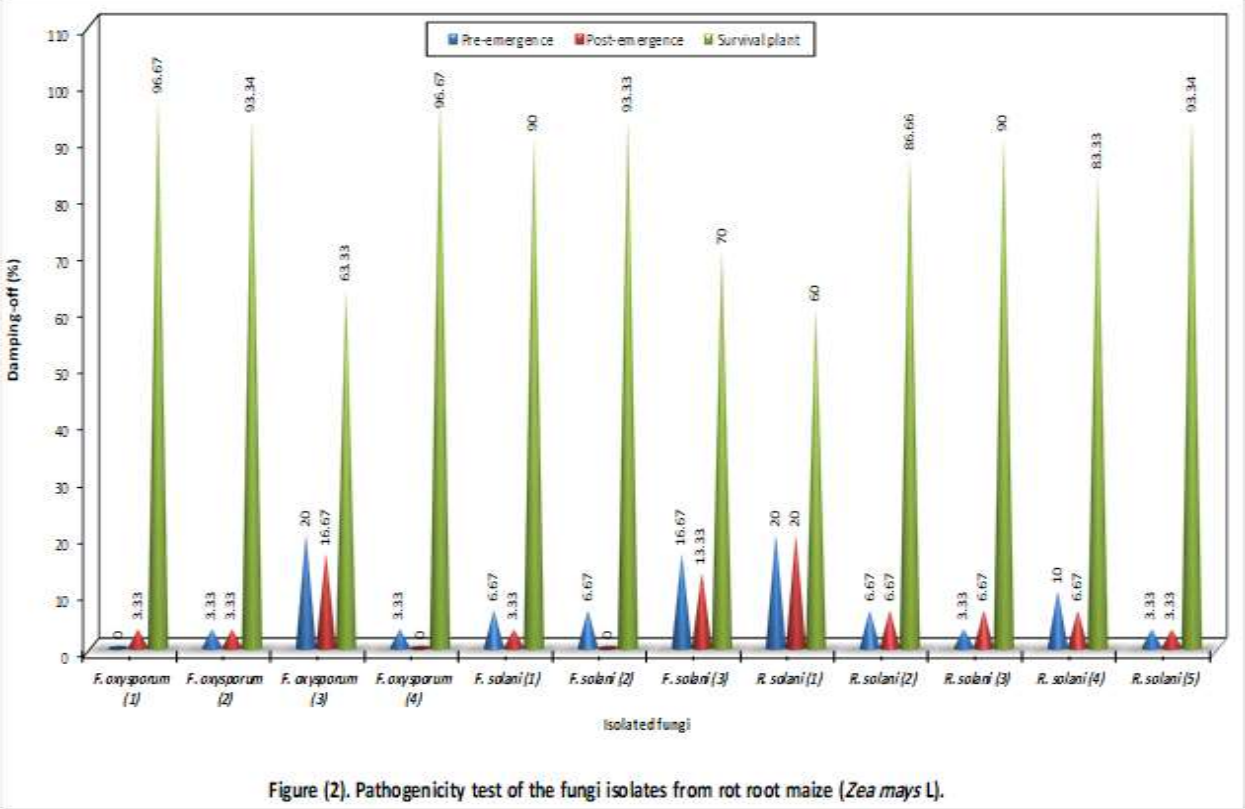

Figure (2). Pathogenicity test of the fungi isolates from rot root maize (*Zea mays* L).

T.harzianum showed a high degree and significant effect of antagonism and diminuend the growth of *F. oxysporum* while *T. konignii* was stronger in decreasing the linear growth of *F. solani* in addition to *R. solani*. The inhibition % of *F. oxysporum* recorded at 66.67% under the application of *T. harzianum*. On the other side, the highest lowering percentage of both

F. solani in addition *R. solani* was observed under the application of *T. Konignii* which recorded 72.3%and 62.44% reduction, respectively. The antagonistic bacteria came an end as compared with antagonistic fungi. It is giving 61.00, 57.55, and 55.55% of inhibition in the linear growth of *F. oxysporum*, *F. solani*, and *R. solani* respectively.

Table (1): impact of examined antagonists on linear growth "mm" of the most pathogenic isolates.

Pathogen / **Antagonists**	***F.oxyporum(3)***		***F.solani(3)***		***R.solani(1)***	
	Linear growth	**Reductio n%**	**Linear growth**	**Reductio n%**	**Linear growth**	**Reduction %**
T.harzianum	**30.00 b**	**66.67**	**28.67 b**	**68.11**	**35.20 b**	**60.89**
T.konignii	**31.00 bc**	**65.55**	**25.17 c**	**72.03**	**32.00 b**	**64.44**
B.subtilis	**35.10 b**	**61.00**	**38.20 b**	**57.55**	**40.00 b**	**55.55**
Control	**90.00 a**	**____**	**90.00 a**	**____**	**90.00 a**	**____**

Means within different letter(s) under the same treatment on each pathogen significantly differ ($p \leq 0.05$).

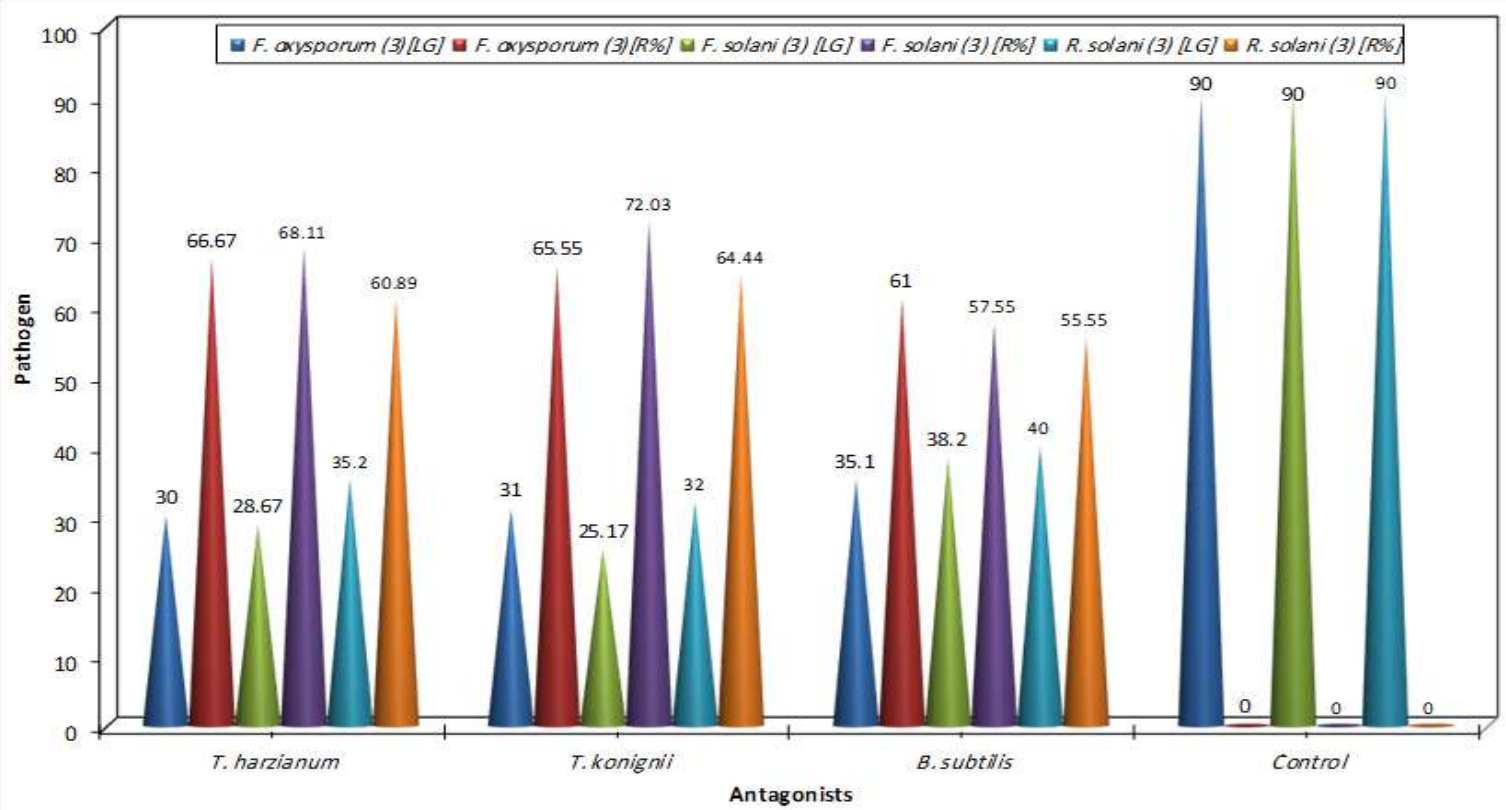

Figure (1). Effect of tested antagonists on linear growth "mm" of the most pathogenic isolates.

(A) *Trichoderma Konignii* and *F.oxysporum.*

(B) Control.

Figure (2): The impact of *Trichoderma konignii* on the fungi *F.oxysporum.*

Figure (3): The impact of *Trichoderma harzianum* on the fungi *F. solaini.*

Figure (4): The effect of *Tirchoderme Konignii* on the fungi *F.solaini.*

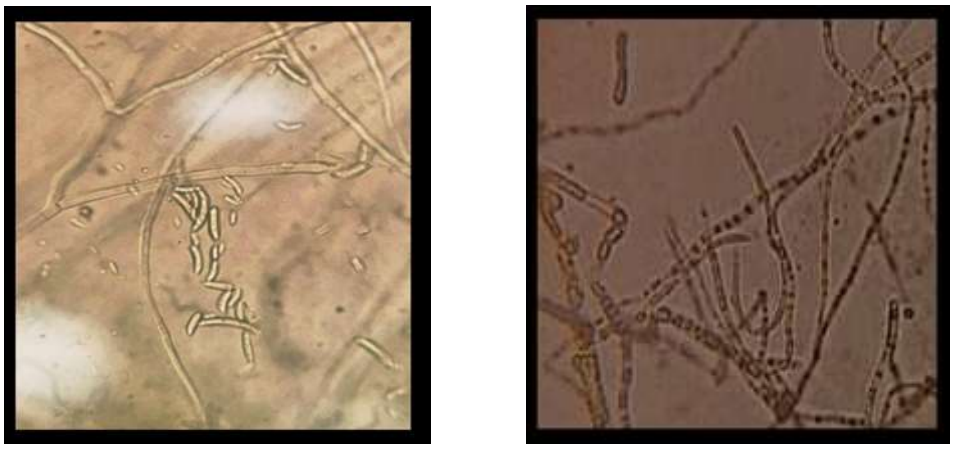

Figure (5): hyphae *Tirchoderme Konignii* on the fungi *F.solaini* X(40).

Figure (6): The effect of *Tirchoderme harzinum* on the fungi *F. oxysporum*.

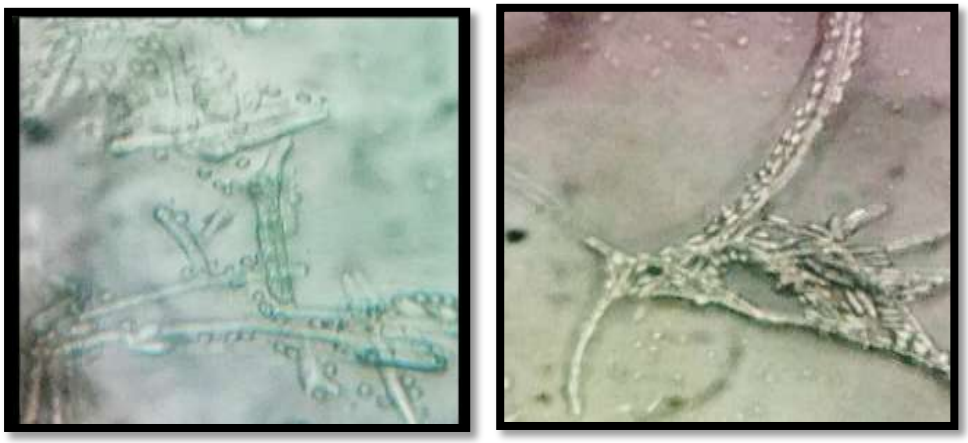

Figure (7): hyphae *Tirchoderme harzianum F. oxysporum* X (40).

Screening of bioagents antagonist, *in vitro:*

The reaction of biotic agents against pathogenic fungi was estimated as Inhibition Zone (IZ), Overgrowth (OG), and Lysis (L) as shown in Table (4). The negative effect was estimated by using *T. harzianum* on IZ, L against *F. oxysporum*, and on OG as well as L on *R. solani*. While it gives of a potential antagonism on the three measures against *F. solani* as well as OG when grows rapidly over the mycelium of *F. oxysporum* and occurs IZ on *R. solani*. It was observed that *T. koningii* also grows considerably faster than three examined pathogenic fungi and occurs lysis in hypha of both *Fusarium spp* viz, F. *oxysporum*, and *F. solani*. On the other side, the antagonistic bacteria i.e. *B. subtilis* lead to the existence of an inhibition zone (IZ) in all tested pathogenic fungi.

Table (2): Mode of action and potential antagonists of the tested bio agents.

Antagonists / Pathogen	*T.harzianum*			*T.koningii*			*B.subtilis*		
	IZ	**OG**	**L**	**IZ**	**OG**	**L**	**IZ**	**OG**	**L**
F.oxysporum	–	+	–	–	+	+	+	–	–
F.solani	+	+	+	–	+	+	+	–	–
R.solani	+	–	–	–	+	–	+	–	–

IZ= Inhibition Zone, OG= Over Growth and L = Lysis

Discussion:-

Utilizing antagonistic microorganisms for biocontrol agents leads to a decrease in the use of the fungicide and minimizes their residues in agriculture products. In addition, it is easy to apply, not expensive, safe, and un-hazardous for humans, and avoids environmental pollution **Abdel-Kader *et al.*, (2013)** It have been discovered that *Trichoderma spp.* Prevent the growth of fungi through three distinct mechanisms: firstly, in order to competitively obtain control of the environment and access to nutrients, secondly, parasitism (deriving nutrients from the host) and thirdly, antibiosis by formation of an inhibitory metabolite or antibiotic **Harman, (2006).** Moreover**, Lorito *et al.*, (1994)** indicated that the mycoparasitism, competition or fungicidal action of *Trichoderma* is due to the ability to from antibiotics or hydrolytic enzymes. The antifungal activity of *T.harzianum* is due to the presence of some metabolites, which are a large group of metabolites and a group also non-volatile, like harzialactones, steroidal antibiotics, *Trichoderma* amide, or anthraquinones **Shakeri and Foster, (2007) and Reino *et al.*, (2008). Nagendra and Kumar, (2011**) stated that the antimicrobial metabolites formed by *Trichoderma spp*. are active against *R. solani.* While **Barakat *et al.*, (2013)** found that, the non-volatile secondary metabolites from the culture filtrate of *Trichoderma spp.* can suppress the mycelial growth of Botrytis fabae than volatile compounds. Moreover, **Saber *et al.*, (2009)** found that *Trichoderma* prevented the linear growth of the pathogen due to produced antibiotics and extracellular enzymes. In addition, **Abd El-Hai and Ali, (2019)** indicated that the application of *T. harzianum* culture filtrates reduced the mycelia growth of peanut root rot pathogenic fungi i.e. *F. solani, R. solani*, and *S. rolfsi.*

Through our study, *T. harzianum* gives off a potential antagonism on the three measures i. e. inhibition zone, overgrowth, and lysis against *F. solani* as well as OG when grows rapidly over the mycelium of *F. oxysporum* and occurs IZ on *R. solani*. It was concluded that *T. koningii* also grows considerably faster than three tested pathogenic fungi and occurs lysis in hypha of both *Fusarium spp* (*F. oxysporum* and *F. solan*i). Moreover, the antagonistic bacteria i.e. *B. subtilis* lead to presence of inhibition zone in all tested pathogenic fungi. These findings are in agreement with which produced by **Saber *et al.*, (2009)** they stated that *Trichoderma spp* grew considerably faster than the pathogen. This

study cleared that, *T.harzianum* displayed high levels and significant effect of antagonism then lowered the growth of *F. oxysporum* while *T. konignii* was stronger in decreasing the linear growth of *F. solani* in addition to *R. solani.*

The variation in one strain's effectiveness towards diversified phytopathogens may be due to high levels of one mechanisms than another, where mycoparasitism is a complex process that causes lytic enzymes to dissolve the fungal cell wall **Reithner *et al.*, (2011).** The direct antagonism of *Trichoderma spp* was showed under the light microscope where observed pathogen mycelium was to be fragmented hyphe, vaculated, and disrupted **Saber *et al.*, (2009)**. They added that after 5 days from the dual culture test, it was discovered that *Trichoderma spp* produced inhibition halos and sporulated over the pathogen colonies. Also, **Melo and Faull, (2000**) and **Harman *et al.*, (2004)** stated that *Trichoderma* produce many necessary enzymes or bioagent; the combined effects of these substances cause parasitism and breakdown of the cell walls, resulting in holes that allow *Trichoderma* hyphae to directly enter the target fungus. Generally, *Trichoderma spp* produces lyases, proteases, lipases, chitinases, and cellulases which acts a role in the decay of pathogen cell walls **Harman *et al.*, (2004).**

Since the antagonistic bacteria can generate antifungal metabolites that can prevent a variety of pathogenic fungi *in vitro*, they are regarded as one of the most significant bio-control agents **Hass and Defago, (2005).** The role of antagonistic bacteria against fungal growth might be due to antibiosis and siderophores production which prevent fungal growth **Whipps, (2001)** and **Shahrak *et al.*, (2009)**. On the other side, *Bacillus subtilis* can make plants resistance to disease by boosting the production of phytoalexin and lytic enzymes **Sailaja and Podile, (1998).**

There are certain strains of *Bacillus* that seems to be active as a biocontrol agent, by preventing the mycelia growth of plant pathogenic fungi **Mahmoud, (2004).** Moreover, **Chung *et al.*, (2008)** found that *B. subtilis* prevented the growth of *F. oxysporum in vitro* by formation of various antibiotics like bacilysin, iturin, or mycobacillin. **Ajilogba *et al.*, (2013)** stated that *B. subtilis, B. cereus, B. pumilus* and *B. amyloliquefaciens* were antagonistic to *F. solani in vivo*. Compiled data regarding the inhibitory impact of different bacteria on soil-borne pathogens were founded by **Abd- El-Kareem *et al.*, (2004).**

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