Impact of Chitosan, *Trichoderma harzianum,* Thyme Oil and Jojoba Extract against Fusarium Wilt Disease of Strawberry

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ABSTRACT: The impact of chitosan, *Trichoderma harzianum,* thyme oil and jojoba extract against *F. oxysporum*, wilt disease of strawberry, was studied in laboratory and throughout the following growing seasons of (2018 and 2019) in greenhouse. The antagonistic biocontrol agent, *Trichoderma harzianum*, was isolated and identified in lab at the Faculty of agriculture Saba Basha, Alexandria, Egypt. In laboratory tests, the effect of the tested biocontrol agents on fungal mean growth *in vivo* was determined. In the greenhouse experiments, the treatments were performed in three application methods: foliar, soil drench and the mixed foliar/soil method. In lab tests, Chitosan® (4g/l) followed by *T. harzianum* and Top perfect® (4ml/l) reduced the fungal growth of *Fusarium oxysporum* pathogen by 86.3, 85.9% and 78.2 %, respectively. In green house experiments, the data showed that the mixed application treatment (soil drench and foliar spray) is important for controlling *Fusarium oxysporum* pathogen. Thyme oil was less effective than the other treatments as biocontrol agent for the pathogen. The Highest determined chlorophyll content was determined in the treatment of *T. harzianum* (48.23), Chitosan® (47.66) and Top perfect® (44.97), without significant differences between *T. harzianum* and chitosan, especially in the mixed application treatment. Moreover, the treatments of Chitosan®, *T. harzianum* and Top perfect®, significantly, showed the highest fresh, dry weights and root length of plants and the least disease incidence and severity percentages compared with the untreated control.

INTRODUCTION

Strawberries are individuals from the family *Rosaceae*, subfamily *Rosoideae*, and genus *Fragaria* (Hummer & Janick, 2009). Strawberries are cultivated in a wide climatic levels, although they are most successfully grown in Mediterranean and temperate climates, which their temperatures ranging between 15 and 30°C in the summer season and between 15 and 20° C in the winter season(Guerrero-Chavez, Scampicchio, & Andreotti, 2015). The sweet strawberry, *F. ananassa*, now dominates strawberry production in nearly all arable regions of the world. Annual strawberry production has increased steadily during most of the 20th century with a slight increase in the rate of growth over the past few decades. The last 20 years have seen production more than double from 3.5 million tons in 1993 to over 8.5 million tons in 2013 (FAOSTAT, 2014). Egypt total area harvested 8880 ha in 2018, in addition to the total of production of strawberries 362639 ton in 2018 (FAOSTAT, 2018). Strawberry is susceptible to numerous soil-borne diseases including root rot, red stele, fusarium wilt, and verticillium wilt. In addition, strawberry is affected by several plant parasitic nematodes. Several diseases of strawberry are caused by pathogen complexes. For instance, root rot disease is caused by a consortium consisting of *Fusarium* spp and *Rhizoctonia fragariae* in association with *P. penetrans* (Particka & Hancock, 2005). Root rot disease produced by many pathogens, as *Fusarium oxysporum*, *Macrophomina phaseolina*, *Pythium* spp. and *Rhizoctonia* spp. (Fang, Finnegan, & Barbetti, 2013). The disease symptom is root death, decline and make black of the main root, and a decay in potency and yield of the plant and significant decrease in the production (Fang, You, & Barbetti, 2012) *F. oxysporum* causes many soil borne diseases as root

and crown rot and wilt disease (Menzies & Jarvis, 1994). These diseases caused high losses of yield amount and quality (Ruiz-Romero, Valdez-Salas, González-Mendoza, & Mendez-Trujillo, 2018). The bioagent *Trichoderma harzianum* achieved a markable inhibition of the plant pathogen, *Fusarium oxysporum,* under *in vitro* condition (Krishna, Nataraj, Rajeshwari, Kirtimala, & Nagaraj, 2019). Thyme (*Thymus vulgaris*) essential oil (TEO) and its ingredients could be used as environmentally friendly biofungicides in the protection of wheat plants against *Fusarium* species (Faghih-Imani, Taheri, & Tarighi, 2020). Chitosan is well-known as bio-control agent because of its nontoxic, biodegradable and biocompatible properties against pathogenic microorganisms by preventing their growth and sporulation (Hassan & Chang, 2017). Now environmental pollution produced by application of chemicals and the random effects of biological control have been commonly evaluated(Zargar et al., 2017). Although pesticides, sometime reaches significant effects, however its effects induced damage of the environment and there organisms. Therefore, the target of this work is to replace the chemical fungicides by other safe approaches as bio-control, which is could be more effective and safe approach.

MATERIALS AND METHODS

The tested biocontrol agents:

Chitosan®: The deacetylated chitin (chitosan) derived from the exoskeleton of shell of crustacean such as shrimp, lobster, crab, squilla, krill, etc., has high economic value owing to its versatile biological activities and agrochemical applications (Badawy et al., 2005). Chitosan is active against viruses, bacteria, fungi, nematodes, and other pests when applied as a foliar or soil treatment, It activated the defense system of the host plant and prevented the invasion of pathogens(El Hadrami, Adam, El Hadrami, & Daayf, 2010). The tested chitosan was a powder form produced by Roth, Germany.

Top perfect® 60%Ec: The formulation contains Jojoba seeds extraction. Jojoba oil, used to control mildew, Jojoba oil is the liquid produced in the seed of the *Simmondsia chinensis* (Jojoba) plant. The oil is structurally different from triglycerides, which are what most of the other seed oils are made of. It has a high shelf life and can be stored for long periods as it is a relatively stable liquid. It does not oxidize easily and will not turn rancid compared to other oils because it does not contain triglycerides (Nimir & Ali-Dinar, 1989). The botanical product was supplied from Top Chemical Company.

Thyme oil: Thyme can refer to plants from the genus Thymus, which is in the family Labiatae, along with the genera Rosmarinus, Lavendula and Salvia. The most widely used species, Thymus vulgaris, is known as common or garden thyme (Morales, 2002). Most thyme oil is produced by steam distillation of the flowering tops of T. vulgaris or T. zygis (Khan & Abourashed, 2011). Thyme oil is rich in the phenolic compound thymol, which is believed to be the main biologically active component (Zarzuelo & Crespo, 2002). Thyme oil was purchased as pure oil from Prof. Dr. Salama EL-Darier, Prof of Plant Ecology and Herbal Medicine, Department of Botany and Microbiology, Faculty of Science, Alexandria University, Egypt.

***Trichoderma harzianum*:** The use of *Trichoderma* species as biological control agents has been investigated for over 70 years but it is only relatively recently that strains have become available commercially. Most isolates of the genus *Trichoderma* that were found to act as mycoparasites of many economically important aerial and soil-borne plant pathogens, have been classified as *T. harzianum* (Gams & Meyer, 1998). The species "*harzianum*" is generally considered as a group made of mycoparasitic and biocontrol strains. The antagonistic potential is the base for effective applications of different Trichoderma strains as an alternative to the chemical control against a wide set of fungal plant pathogens (Harman, 1998).

A. Laboratory tests: Four biocontrol agents namely Chitosan®, Top perfect®, thyme oil and *Trichoderma harzianum* were evaluated against the plant pathogen, *Fusarium oxysporum* under laboratory conditions. Three concentrations were tested for each of Chitosan®, Top perfect®, thyme oil (Table, 1).

Table (1). Materials and their concentration that used in the laboratory studies

Biocontrol agent	Concentration
Chitosan® powder	1.0, 2.0 and 4.0 g /L
Top perfect® 60% Ec	1.0, 2.0 and 4.0 ml /L
Thyme oil	1.0, 2.0 and 4.0 ml /L
Trichoderma harzianum	$1x10^7$ spore/ml

Isolation of the pathogen:

Samples of infected strawberry plants were taken from Abo-elkhaw village, Komhamada, El-Beheira Governorate, Egypt. The roots were rinsed, and dried by air, the samples were sterilized in 1% sodium hypo chloride surface sterilized for three minutes, rinsed by sterilized distilled water many times and put it between 2 sterilized filter papers to make dried. The sterilized samples were moved to plates containing potato Dextrose agar medium (PDA). Petri-dishes were incubated at 25°C for 3 days. The progress of hyphal growth was picked up and moved onto new Petri-dishes. Purification of the isolated fungus was carried out using the hyphal tip technique followed by (Hawker, 1956) . Identification of the isolated fungus was approved by their cultural and morphological characteristics defined by (Barnett & Hunter, 1987). Then, the stock cultures was kept on PDA slants and saved in the refrigerator at 5°C.

Isolation of the antagonistic microorganisms:

Healthy roots of strawberry were collected from strawberry field. Their rhizospheric soils were utilized for collecting many antagonistic microorganisms by the technique defined by (Ahmed, 2005). One gram of rhizospheric soil was put into 99 ml sterilized water; shacked 15 minutes and serial dilutions up to 10^{-4} were prepared. Autoclaved potato dextrose agar + rose Bengal + streptomycin medium (Johnson, Curl, Bond, & Fribourg, 1959) was used for isolating the antagonistic fungi. One ml of each dilution was aseptically transferred to sterilized Petri-dishes; containing 10 ml of melted warm agar medium and three plates were used for each dilution. All plates were incubated at 25±1°C for 4

days. Identification of the isolated fungus was approved followed by their cultural and morphological characteristics defined by(Schleifer, 2009). Identification was confirmed at the Faculty of agriculture Saba Basha, Alexandria, Egypt.

Evaluation of *Trichoderma harzianum* as a biocontrol agent against *Fusarium oxysporum* by the dual culture technique

The antagonistic isolate of Trichoderma harzianum was evaluated against *F. oxysporum* by dual culture technique as described by Dennis and Webster (1971). Five mm diameter mycelial discs of the *Trichoderma* culture and *F. oxysporum* were placed opposite to each other in the petri plates at equal distance from the periphery. The antagonists were inoculated at one side of petri dish containing PDA. Discs from the culture of *F. oxysporum* were placed at the opposite side of petri dishes perpendicular to the antagonists and incubated at 28^{o}C. Petri dishes inoculated with fungal pathogen discs alone served as control. Three replications were maintained for each isolate. Radial growth of *F. oxysporum* isolates was recorded and inhibition percent of pathogen growth was calculated by using the following formula:

$$Inhibition(\%) = \frac{C - T}{C} \times 100$$

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual culture plate

Evaluation of chitosan, thyme oil and formulation of jojoba oil as biofungicide against *F. oxysporum*

Chitosan solution was prepared by dissolving the chitosan in 0.1 N acetic acid and diluted in distilled water with pH adjusted at 6.5. Chitosan solution were prepared at three diverse concentrations 0.0, 1.0, 2.0 and 4.0 g /L followed by (Benhamou & Bélanger, 1998). Thyme oil was added at 0.0, 1.0%, 2.0%, and 4.0% (v/v) concentration. Top perfect (jojoba oil) was used at concentrations of 0.0, 1.0, 2.0 and 4.0 ml /L. Each treatment concentration was put into warm sterilized PDA medium and was shacked before transferred into Petri dishes (10 ml/plate) mycelial disks (5 mm in diameter) from the (*Fusarium oxysporum*) were placed in the center of 90-mm Petri dishes containing 10 mL PDA. Control Plates contained media only. Then were used 3 plates for each treatment. Incubated the Petri-dishes was at 25±1°C. Radial growth of *F. oxysporum* isolates was recorded and per cent inhibition of pathogen growth was calculated by using the formula.

$$Inhibition(\%) = \frac{C - T}{C} \times 100$$

Where, C- mycelial growth of pathogen in control

T- mycelial growth of pathogen in dual culture plate

B. Greenhouse experiments:

Treatments and experimental design Completely randomized design was followed in the experiment with three replications. Experiment conducted under greenhouse conditions at Fac. of Agriculture saba basha, Alexandria University in two season 2018-2019. Plastic pots 20 cm diameter were sterilized by dipping in 5% formalin solution for 5 min and then left to dry. Disinfected sand soil (with 5% formalin) was distributed in plastic pots. Each pot was injected with

10 ml of Fusarium culture suspension (10^7conidia/ml) (El-Khallal, 2007)**.** Soil infestation was performed 7 days before planting strawberry. Festival cultivar of strawberry transplants acquired from Horticulture Institute, ARC, Giza. Transplants were transplanted in the potted infested or non-infested (control) soil at the level of one transplant per pot and each replicate contained three plants in three pots. pots were kept under greenhouse conditions. Strawberry plants were examined periodically (every week for seven weeks). Percentages of dead plants show root rot symptom were recorded to determine virulence of the pathogen. Treatments were organized in a completely randomized design. Prepared chitosan solution by dissolving the chitosan in 0.1 N acetic acid and diluted in distilled water with pH adjusted at 6.5. Chitosan solution was prepared at 4.0 g/L concentrations (Benhamou & Bélanger, 1998), Essential oil, *i.e*., thyme at concentration of 0.4% (v/v), Top perfect (jojobaoil) was used at concentration of 4.0 ml /L, *Trichoderma harzianum* was used at concentration of (10^7 spore/ml). Each treatment and a control were evaluated. Every treatments were applied as soil drench, foliar spry and mixing (soil drench and foliar spry). In each treatment data were recorded as root length, chlorophyll index, fresh weight, dry weight, disease incidence percentage and Disease severity percentage.

Disease assessments: In greenhouse experiment, percentage of disease incidence of root rot diseases were determined (49 days from transplanting) post transplanting according to the method described by (Ahmed, 2005) as following :-

$$\text{Disease incidence \%} = \frac{\text{Number of infected planta}}{\text{Total number of plants}} \times 100$$

Disease severity (DS) was recorded at the end of experiment according to original scale developed to evaluate disease according to (Ahmed, 2005) as follows: 0=no symptoms, 1=1-10% plants yellowing; 2=1-25% plants yellowing; 3=26-50% wilting and yellowing; 4=51-100% dead plants.

$$\text{Disease severity \%} = \frac{\sum(\text{disease grade} \times \text{number of plants in each grade})}{\text{Total number of plants} \times \text{highest disease grade}} \times 100$$

Fresh and dry weights of the plants were assessed separately for shoot and root system according to the analytical procedure developed by (Ostrowska, Gawliński, & Szczubiałka, 1991). The intensity of the green color of the leaves was measured with the use of a SPAD tool, used for determination of intensity of green color of the leaves (highly correlated with chlorophyll content in the leaves)(Ahmed, 2005).

Statistical analysis: The data were elaborated statistically by ANOVA and significance of the differences between the treatments were evaluated by Student-Newman- Keuls multiple range tests at p = 0.05.

RESULTS AND DISCUSSION

Isolation and identification of Fusarium pathogen

Identification of the isolated fungal cultures from El-Beheira governorate followed according to cultural and morphological characteristics**.** The isolates were identified as *Fusarium oxysporum*. Identification was carried out at

Laboratory of Plant Pathology, Fac. of Agriculture (Saba-Basha), Alexandria University, Egypt.

Effect of the treatments on fungal growth *in vivo*

This experiment aimed to evaluate the antagonistic effect of the studied treatments on controlling *Fusarium oxysporum* pathogen, in addition to select the suitable treatments for *in vivo* experiments. Results presented in Table, 2 and illustrated in Figure 1 and 2 showed that all evaluated treatments significantly reduced hyphal growth rates of fusarium. However, they significantly varied in their antagonistic potentials. The highest mean values of growth reduction were recorded by Chitosan® at concentration 4g/l (86.3%), followed by *T. harzianum* (85.9%) and Top perfect® at concentration 4ml/l **(**78.2 %), However, the lowest antagonistic effect against the tested isolate was obtained by thyme oil at concentration 4ml/l (13.3%).

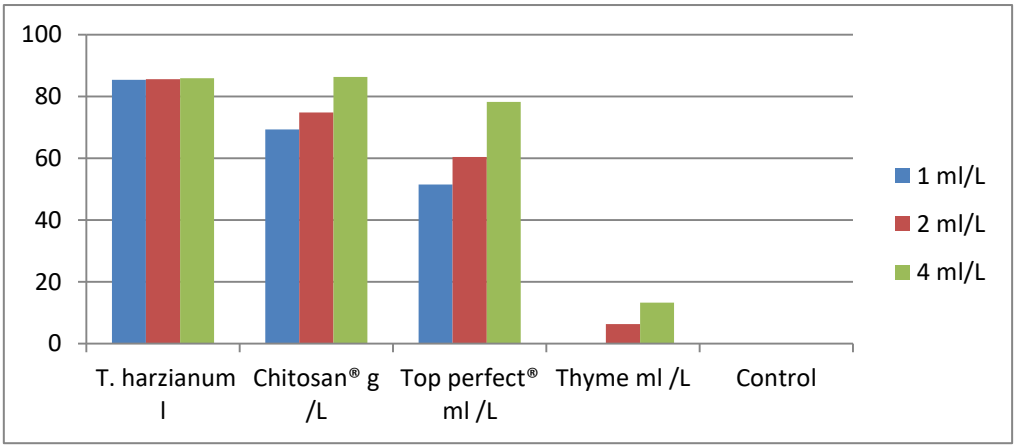

Figure (1). Efficacy of each treatment on fungal growth *in vitro*

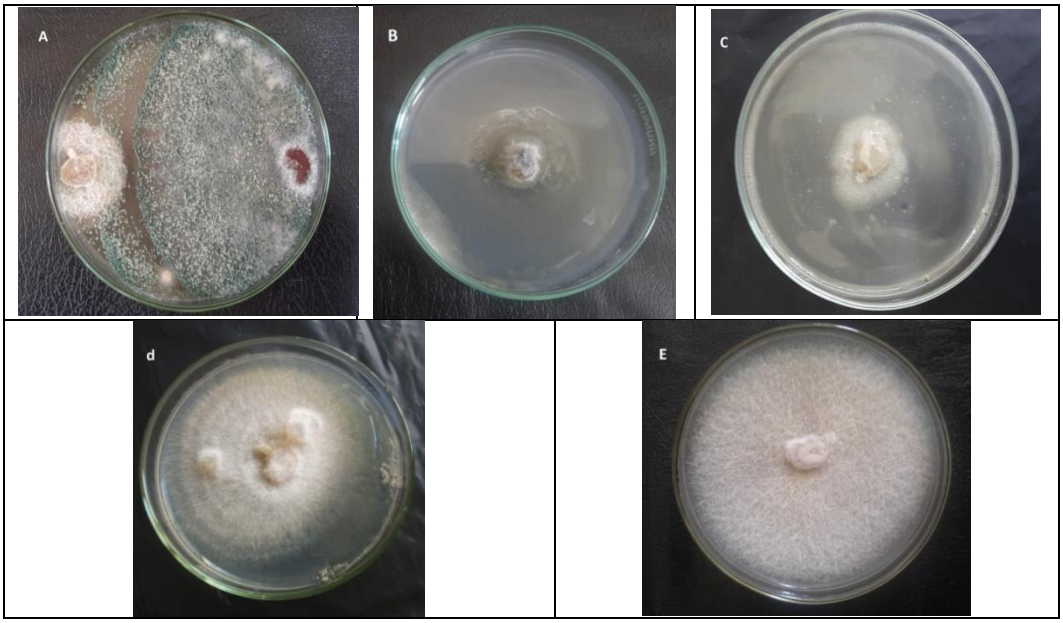

Figure (2). Antagonistic effect of the four tested bio-control agents on *Fusarium oxysporum*. Where: A= *T. harzianum*, B= Chitosan, C= Top perfect,D= thyme oil, E= untreated control

Table (2). Efficacy of each treatment on *F. oxysporum* growth *in vitro*

Treatments	Reduction% Concentrations g/l			General average
	1	2	4	
T. harzianum	85.4 a	85.6 a	85.9 a	**85.6 a**
Chitosan® g /L	69.3 d	74.8 c	86.3 a	**76.8 b**
Top perfect® ml /L	51.5 hi	60.4 fg	78.2 b	**63.3 d**
Thyme ml /L	0.0 m	6.3 l	13.3 k	**6.5 h**
Control	0.0 m	0.0 m	0.0 m	**0.0 i**
General average	**44.1 c**	**52.4 b**	**59.8 a**	

LSD0.05 for Treatments =1.17 LSD0.05 for concentrations= 0.67

Effect of the treatments on fusarium disease under greenhouse conditions

During the first season 2018:

The results presented in table 3 and Fig. 3 show the efficacy of different treatments against *F. oxysporum*. Every treatment was applied as soil application (soil drench) and as foliar spry. Data were recorded after 7 weeks of transplanting to describe the effect of treatments on the *F. oxysporum*. whereas, the treatments may be effect on the plant components like that the chlorophyll. *T. harzianum* was the highest effective treatment concerning to chlorophyll for soil drench and mix between soil and foliar sprays application (45.77 and 48.23). In addition, the obtained data cleared that no significant difference between both of *T. harzianum* or Chitosan® (47.66) and Top perfect® (41.6) after mixed application. By comparing the effect of three methods of application the highest mean effect on the chlorophyll accrued by the mixed application (42.41) followed by injection method (39.33) and foliar spray (35.93), respectively.

Table (3). Efficiency of the treatments on chlorophyll index during season 2018

Treatments	Chlorophyll index Type of application methods			General average
	Spray	Soil	Mix	
T. harzianum	43.13 efgh	45.77 bcde	48.23 a	**45.71 a**
Chitosan® g /L	41.83 h	45.3 bcdefg	47.66 ab	**44.93 a**
Top perfect® ml /L	36.86 i	43.03 fgh	44.97 cdefg	**41.62b**
Thyme ml /L	19.3 k	35.4i	37.47i	**30.72c**
Control	19.6 k	19.56 k	19.36 k	**19.51d**
General average	**35.93c**	**39.33 b**	**42.41a**	

LSD0.05 for Treatments= 0.94 LSD0.05 for application methods=0.54

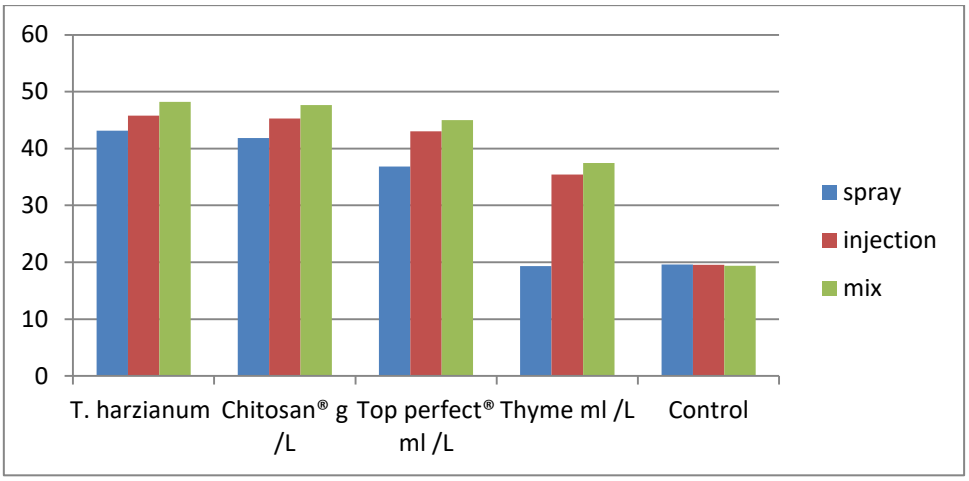

Figure (3). Efficiency of the treatments on chlorophyll index during season 2018

Data presented in table 4 and Fig 4 and 5 indicate that all tested compounds significantly increased fresh and dry weight of plants in the first growing seasons. The highest fresh weight mean achieved by chitosan as mixed treatment (34 gm) followed by *T. harzianum* (30.1gm). The highest mean number accrued by mix application 6.7 a followed by injection (5.2gm) and spray application (3.8gm), respectively. In addition, chitosan recorded the highest mean weight of the dry weight by the mixed application (8.6gm) followed by *T. harzianum* (8.1 gm), respectively.

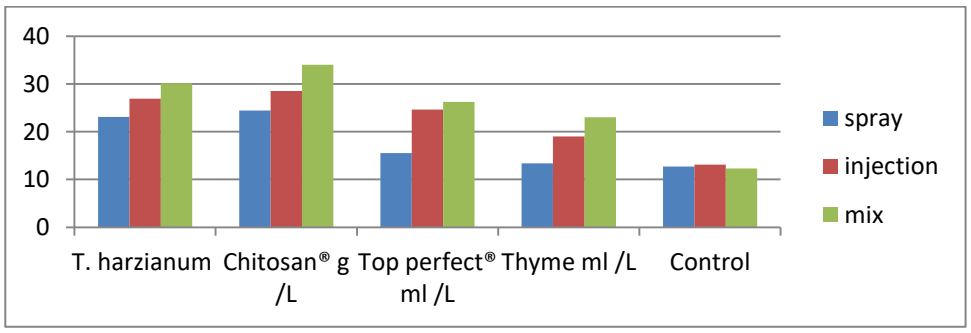

Figure (4). Efficiency of tested biocontrol agents on Strawberry plants (fresh weight) infested by *F. oxysporum* during season 2018

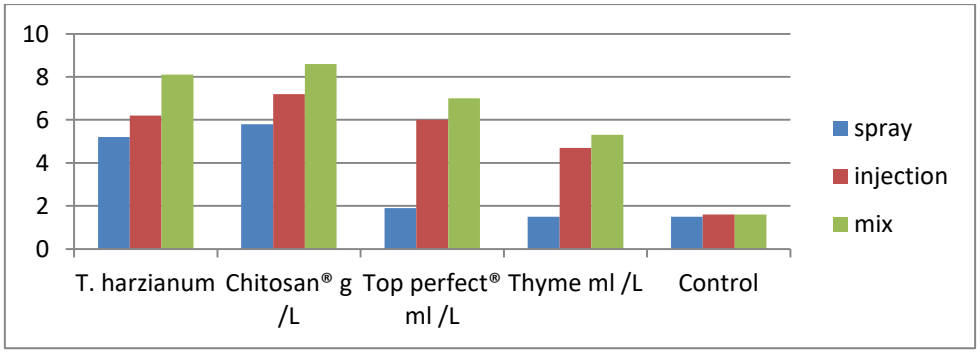

Figure (5). Efficiency of tested biocontrol agents on Strawberry plants (dry weight) infested by *F. oxysporum* during season 2018

Table (4). Efficiency of tested biocontrol agents on Strawberry fresh and dry weight infeced by *Fusarium oxysporum* during season 2018

Treatments	Fresh weight (g)				Dry weight (g)			
	Type of application methods			General average	Type of application methods			General average
	Spray	soil	Mix		Spray	soil	Mix	
T. harzianum	23.1ef	26.9cd	30.1b	26.8b	5.2hijk	6.2 defg	8.1ab	6.5b
Chitosan® g /L	24.4de	28.5c	34 a	29a	5.8 fghi	7.2 c	8.6 a	7.2a
Top perfect® ml /L	15.5h	24.6de	26.2c d	22.1f	1.9 i	6 fgh	7cd	5e
Thyme ml /L	13.4hij	19g	23 ef	18.4g	1.5 i	4.7k	5.3hijk	3.8f
Control	12.7ij	13.1ij	12.3 l	12.7h	1.5 i	1.6 i	1.6 i	1.6g
General average	19.7c	22.6b	26.7a		3.8c	5.2 b	6.7a	

LSD0.05 for Treatments 1.01
LSD0.05 for application methods 0.58
LSD0.05 for Treatments 0.34
LSD0.05 for application methods 0.19

Table (5).Efficiency of the treatments on(disease incidence and severity) the plants infested by *Fusarium oxysporum* during season 2018

Treatments	Disease incidence %			General average	Disease severity %			General average
	Type of app. methods				Type of app. methods			
	Spray	soil	Mix		Spray	soil	Mix	
T. harzianum	29.6ef	22.2efgh	11.1hij	20.97c	16.7f	11.1gh	8.3hi	12.1e
Chitosan® g /L	18.5fghi	11.1hij	7.4ij	12.4e	11.1gh	6.5i	2j	6.5f
Top perfect® ml /L	33.3de	25.9efg	18.5fghi	25.9cd	27.8e	13g	10.2gh	17d
Thyme ml /L	100a	48.2bc	40.7cd	62.96b	100a	43.5c	33.3d	58.9b
Control	100a	100a	100 a	100a	100a	100a	100a	100a
General average	44a	32.5b	28.4c		38.9a	24.5b	19.4c	

LSD0.05 for Treatments =4.52
LSD0.05 for application methods= 2.61
LSD0.05 for Treatments= 1.31
LSD0.05 for application methods= 0.75

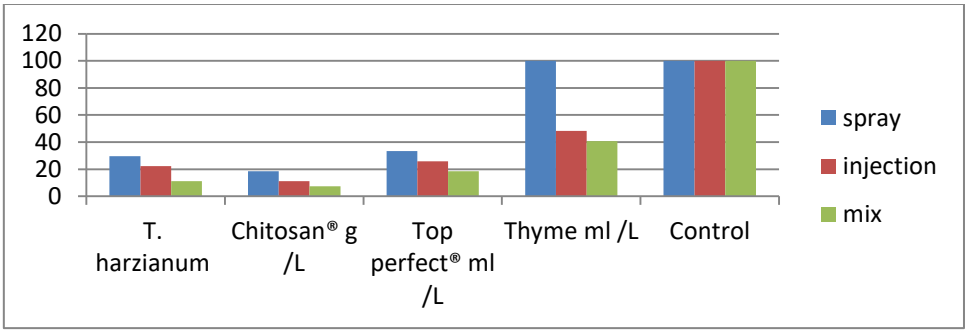

Figure (6). Efficiency of the treatments on disease incidence during season 2018

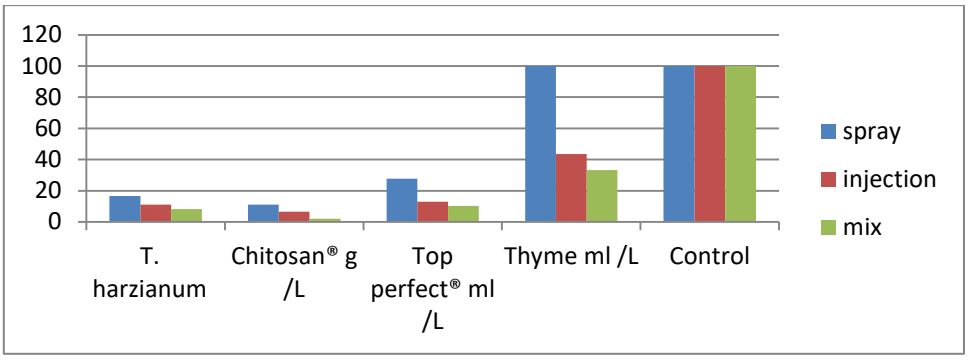

Figure (7). Efficiency of the treatments on disease severity during season 2018

Disease incidence (DI %)

The obtained results in Table 5 and Fig.6 showed that all treatments reduced disease incidence and severity significantly in the first seasons. In addition, all application methods achieved high reduction of disease incidence compare with control. The highest reduction disease incidence recoded by chitosan® after mix and soil applications (7.4 % and 11.1%) followed by Trichoderma (20.97 %), top perfect® (25.9%). Also, when compared between mean number of application methods for disease incidence. Moreover, the highest DI % value was detected in control mean (100%), followed by thyme oil mean (62.96%). Chitosan® treatment mean achieved the highest reduction of disease incidence (12.4 %).

Disease severity (DS %)

According to results presented in Table 5 and Figure 7, variances in DS percentages among the evaluated treatments were, generally, significant. The highest DS% was achieved on control followed by thyme oil treatment (100% and 58.9%), sequentially. on the other hand, the most effective treatment of reducing the DS percentages observed by chitosan® (5.6%) followed by Trichoderma (12.7%) and top perfect® (17%). In addition, all application methods achieved high reduction of disease incidence compare with control. The highest reduce effect on disease incidence found by chitosan® after mix and soil application (2 % and 6.5%). Also, the same trend was observed when compared with mean number of application methods for DS percentages. We found that the highest number for application methods accrued by mix (soil + spray) application (19.4%).

Effect of biocontrol agents on root length (cm)

The results presented in table 6 and Fig 8 reported that all treatments increased the root length of plants when compared with control. The highest mean number of root length recorded by chitosan (28.3 cm) followed by *T. harzianum*. When compared with the mean number of treatments the highest mean number of root length occurred by chitosan (25.6 cm) followed by *T. harzianum.* Nevertheless, when compared with the mean number of application methods the highest mean number occurred after mix application (24 cm).

Table (6). effect of tested biocontrol agents on root length of plants infested by *Fusarium oxysporum* during season 2018

Treatments	Root length cm			General average
	Type of application methods			
	Spray	Soil	Mix	
T. harzianum	20 g	25 cd	27.7 ab	24.2 b
Chitosan®	22 f	26.3 bc	28.3 a	25.6 a
Top perfect®	19.3 gh	25.3 cd	25.7 bcd	23.4bc
Thyme	12.6 k	15.8 j	17.9 h	15.4 d
Control	12 jk	11 k	10.7 k	11.2 e
General average	18.9 c	21.1 b	24a	

LSD0.05 for Treatments =0.94
LSD0.05 for application methods=0.54
LSD0.05 for Interaction between Treatments and application methods = 1.62
* Means followed by the same letter(s) in each column are not significantly different at P ≤ 0.05 level

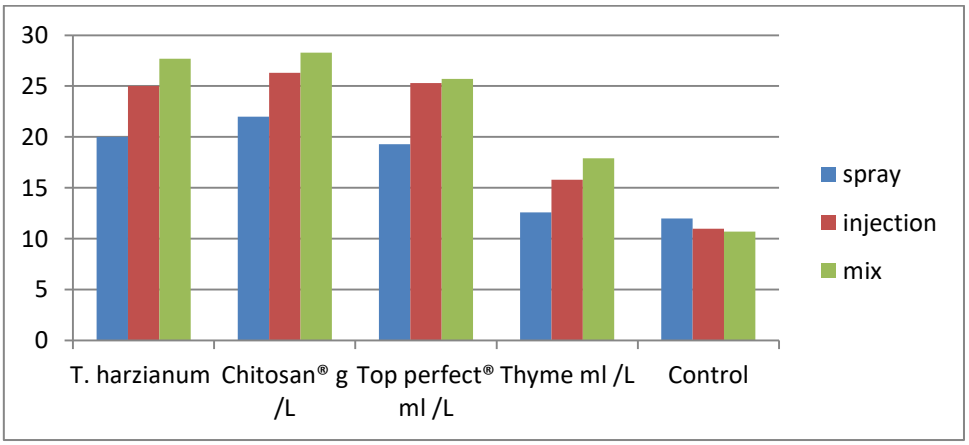

Figure (8). Effect of the treatments on root length during season 2018

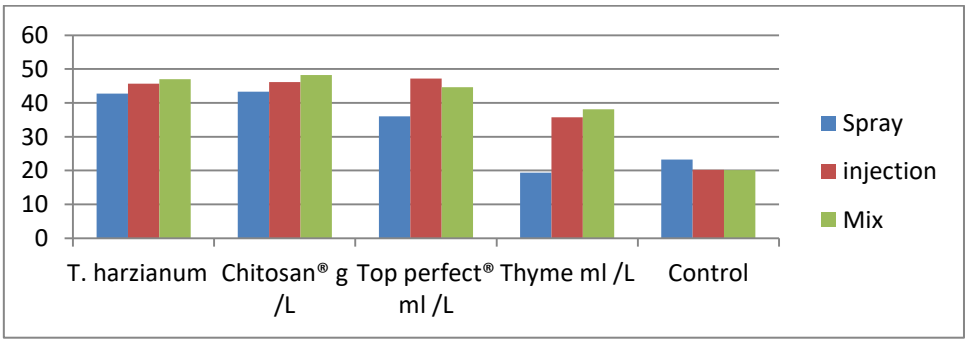

Figure (9). Effect of tested biocontrol agents on chlorophyll index on plants infected by *Fusarium oxysporum* during season 2019

Data existed in Table 7 and Fig. 9 showed that chitosan® was the highest effective treatment concerning on "chlorophyll for mix application (48.2). In addition, the result were cleared that no significant difference between the chitosan® and *T. harzianum* (47) with in the mixed application. The result

showed that when compared between application methods, the highest effect on the chlorophyll observed by mix application (42.5) followed by soil application method (39.6) and foliar spray (35.1).

Table (7). effect of the treatments on chlorophyll index during season 2019

Treatments	Chlorophyll index			General average
	Type of application methods			
	Spray	Soil	Mix	
T. harzianum	42.7 f	45.7 bcd	47 b	45.1 ab
Chitosan® g /L	43.3 ef	46.1 bc	48.2 a	45.9 a
Top perfect® ml /L	36 h	47.2 ab	44.6 cde	41 d
Thyme ml /L	19.4 k	35.7 h	38.1 g	31.1 f
Control	23.2 i	20.2 jk	20.1 jk	21.2 g
General average	35.1 c	39.6 b	42.5 a	

LSD0.05 for Treatments =0.73 LSD0.05 for application methods=0.42

Data illustrated in Table 8 and Fig 10 and 11 indicated that all tested compounds significantly increased fresh and dry weight of plants. In this regard, the highest efficacy on mean fresh weight achieved by *T. harzianum* and chitosan on mix treatment recorded (33.9 g), (33.5 g), respectively. The highest mean number obtained by mix application (6.3 g) followed by injection (5.3 g) and foliar spry application (3.8 g), respectively. Also, when determined the dry weight after mix application *T. harzianum* recorded the highest mean number (8.4 g) followed by chitosan® (7.9 g).

Table (8). Effect of the treatments on fresh and dry weight during season 2019

Treatments	Fresh weight (g)			General average	Dry weight (g)			General average
	Type of application methods				Type of application methods			
	Spray	Injection	Mix		Spray	injection	Mix	
T. harzianum	23.1fg	26.2cde	33.9a	27.7a	5.1ghi	6.6cd	8.4a	6.7a
Chitosan® g/L	24.8def	28.3bc	33.5a	28.5a	5.8defg	6.7cd	7.9ab	6.8a
Top perfect® ml /L	15.3i	24.3ef	27.4cde	22.3d	1.8 j	6.2 cdef	6.5 cd	4.8 d
Thyme ml /L	12.7 j	18h	23.3fg	18e	1.5 j	4.9 ghi	5.6 defghi	4 e
Control	11.9 j	12.3 j	12.6 j	12.3f	1.6 j	1.4 j	1.4 j	1.5 f
General average	18.9 c	22.7b	26.9 a		3.8 c	5.3 b	6.3 a	

LSD0.05 for Treatments =1.13
LSD0.05 for application methods= 0.65

LSD0.05 for Treatments =0.44
LSD0.05 for application methods= 0.25

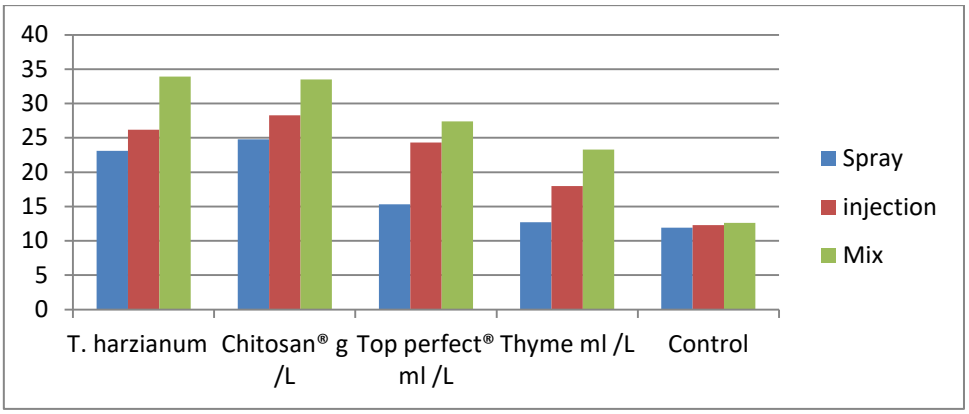

Figure (10). Effect of the treatments on fresh weight of plants during season 2019

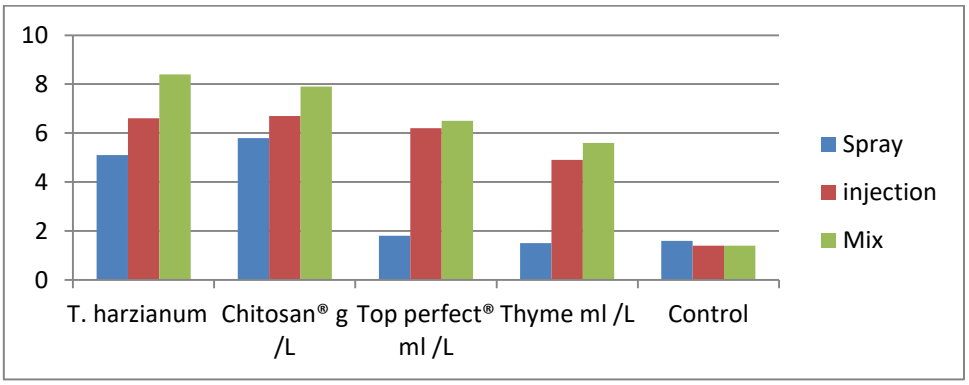

Figure (11). Effect of the treatments on dry weight of plants during season 2019

Table (9). Effect of the treatments on disease incidence and disease severity 2019

Treatments	Disease incidence % application methods			General average	Disease severity % application methods			General average
	Spray	soil	Mix		Spray	soil	Mix	
T. harzianum	22.2defg	22.2defg	11.1ghi	18.5d	16.7e	11.1fg	7.4hi	11.7d
Chitosan® g /L	14.8fghi	11.1ghi	7.4hi	11.1e	11.1fg	7.4hi	1.9j	6.8e
Top perfect® ml /L	33.3cd	25.9def	25.6de	29.6c	33.3d	13f	11.1fg	19.2c
Thyme ml /L	100a	51.9b	40.4c	64.2b	100a	43c	33.3d	58.6b
Control	100a	100a	100a	100a	100a	100a	100a	100a
General average	39.5a	24.5b	25.9c		39.5a	24.5b	19.5c	

LSD0.05 for Treatments 4.67
and for application methods 2.69

LSD0.05 for Treatments 0.97
and for application methods 0.5

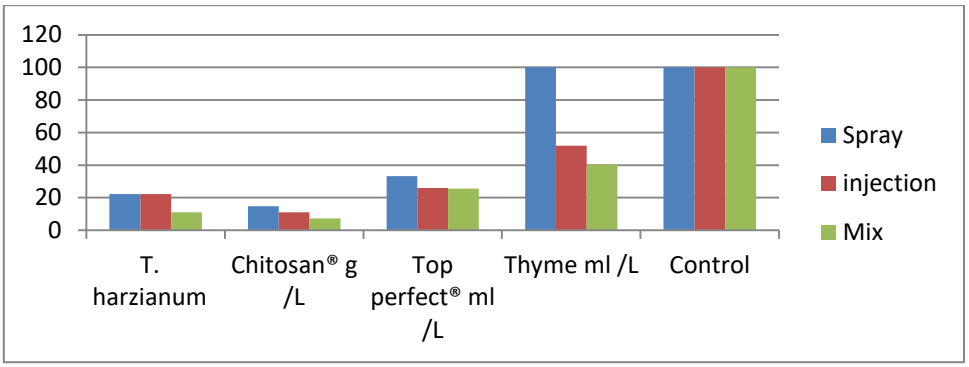

Figure (12). Effect of tested biocontrol agents on disease incidence of plants infected by *Fusarium oxysporum* during season 2019

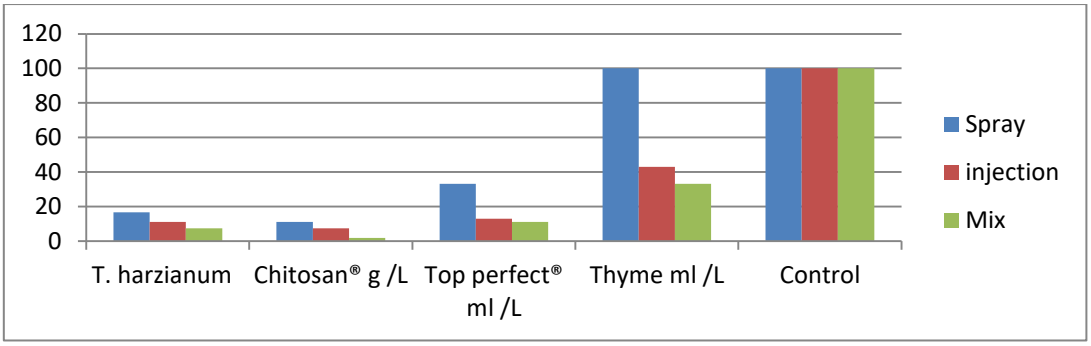

Figure (13). Efficiency of tested biocontrol agents on Strawberries (disease severity) infected by *Fusarium oxysporum* during season 2019

Disease incidence and disease severity

The obtained results showed in Table 9 and Fig. 12 and 13 cleared that all treatments significantly reduced disease incidence and severity in both growing seasons. The highest reduce disease incidence accrued by Chitosan® after mix and soil application (7.4 % and 11.1 %) followed by *T. harzianum*(11.1%) by mix application. The same result obtained on disease severity by chitosan (1.9 %). Also, the mixed application method showed the most effective in decreasing disease incidence and severity to only 25.9 % and 19.5 %, respectively compared with the other application methods.

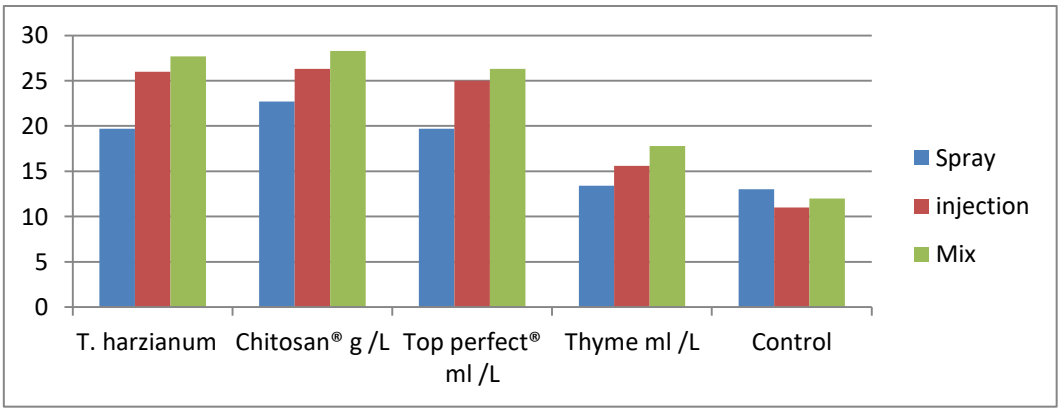

Figure (14). Effect of tested biocontrol agents on Strawberry (root length) infested by *Fusarium oxysporum* during season 2019

Table (10). Effect of tested biocontrol agents on Strawberry (root length) infested by *Fusarium oxysporum* during season 2019

Treatments	Root length (cm)			General average
	Type of application methods			
	Spray	soil	Mix	
T. harzianum	19.7 fg	26 bc	27.7 ab	24.4 b
Chitosan® g /L	22.7 e	26.3 abc	28.3 a	25.8 a
Top perfect® ml /L	19.7 fg	25 cd	26.3 abc	23.7 bc
Thyme ml /L	13.4 i	15.6 h	17.8 g	15.6 e
Control	13i	11j	12ij	12 f
General average	18.8 c	21.6 b	24.2 a	

LSD0.05 for Treatments 0.81 LSD0.05 for application methods0.47

During season 2019, the results showed in (Table 10 and Fig. 14) reported that all treatments increased the root length Strawberries when compared with untreated control. The highest mean number of root length recorded by chitosan (28.3%) followed by *T. harzianum* (27.7 %). When compared with between the mean number of treatments the highest mean number of root length accrued by *chitosan* (25.8 %) followed by *T. harzianum.* However, when compared with the mean number of application methods the highest mean number occurred on mix application (24.2 %)

DISCUSSION

Effect of the treatments on fungal growth *in vitro*

The highest mean values of fungal growth reduction were induced by Chitosan (86.3%) at concentration 4g/l, followed by *T. harzianum* (85.9%) and Top perfect® **(**78.2 %). However, the lowest antagonistic effect against the tested isolates was obtained by thyme oil (13.3%). Similar results were mentioned by (Li, Sun, Yang, Ge, & Yi, 2009) who studied the antifungal activity of chitosan on *Fusarium* spp. causing dry rot of potato tuber, High reduction of mycelial growth of *Fusarium* spp. achieved by the application of chitosan at higher concentration causing more damage to fungal hyphae. As well, *In vitro* treatments of CH + SA in PDA medium caused the maximum inhibition in the linear growth and spore germination of *F. solani* incubated plates compared to control plates. Moreover, (Krishna et al., 2019)) evaluated the effect of six bio agents against *F. oxysporum* under *in vitro* condition. *T. harzianum* was more effective against the pathogen and exhibited (90.06 %) percent inhibition. In addition, (Redda et al., 2018) evaluated the antagonistic efficacy of *Trichoderma* spp. against *F. oxysporum* and found strong antagonistic potential which inhibited >50% linear growth of *F. oxysporum*. Furthermore, (Suleiman, Gambo, & Sunusi, 2019) reported that *T. harzianum* isolates had the highest effect of inhibition the linear growth of the pathogen *F. oxysporum* compared to control *in vitro.*

Antagonistic effects of evaluated treatments on strawberry root rot disease

The results proved that all treatments achieved high reduction of disease incidence and severity. In addition the tested treatments increased the chlorophyll index, total fresh weight , dry weight and root length of strawberry

plants agree with our obtained results (Mukta *et al*., 2017) studied the efficiency of chitosan and fungicides on strawberry production. Chitosan @ 250–500 ppm was applied on Strawberry Festival achieved 56% higher production compared to control and Fungicide application. (El-Mohamedy, Shafeek, Abd El-Samad, Salama, & Rizk, 2017) results indicated that soaking bean seeds in CH1.0g/l + SA 5% trailed by spray application, caused in the highest inhibition of damping-off and root rot incidence 70.0%, compared with control. As well (Sinha, Harshita, Singh, & Verma, 2018) evaluated *T. harzianum* and *T. viride* for their efficacy against *F. oxysporum* in green house conditions. *T. harzianum*has also achieved the highest germination along with improved plant height, root length and yield. (Khan et al., 2017)Found that *T. harzianum* achieved 75.5% inhibition of colony growth of *F. oxysporum* pathogen followed by incubation for 72 h at 28+2°C*in vitro*. Furthermore the reduction of disease severity on pot experiment. More ever (Moosa *et al*., 2017) reported that *F. oxysporum* causing wilt disease, affecting huge losses in yield. They evaluated the effects of the antagonistic isolates of *T. harzianum* against *F. oxysporum*. Trichoderma isolates achieved the highest reduction best on Fusarium wilt of tomato. Furthermore (Ahmed & El-Fiki, 2017) found that *Trichoderma* spp. The highest growth reduction of tried pathogen *F. oxysporum* was occurred by *T. harzianum*(74.67 mm). Moreover application of *Trichoderma spp*. achieved high increase in phenols, nitrogen and chlorophyll index. On other hand (Mansour & El-Sharkawy, 2014) evaluated Jojoba (*Simmondsi achinensis*) oil activity on Squash root rot disease that caused by *R. solani* and *F. solani*. The results indicated to the reduction of damping- off and dead plant compared with untreated seeds. Squash seeds treated with Jojoba oil increased fruit yield/plot and plant survival compared with control. As well (Baraka, Radwan, Shaban, & Arafat, 2011) evaluated the efficacy of jojoba as fixed oil at 500 ppm against root rots pathogen of date palm tree caused by *F. oxysporum* and many fungi. The result indicated that jojoba as fixed oil and other essential oils have most reduction of the plant pathogen.

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الملخص العربي

تأثير الشيتوزان والتريكودرما وزيت الزعتر ومستخلص الجوجوبا ضد مرض الذبول الفيوزارمي للفراولة

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تأثير الشيتوزان والتريكودرما وزيت الزعتر ومستخلص الجوجوبا ضد مرض الذبول الفيوزارمي على محصول الفراولة تم دراسته معمليا و في الصوبة خلال الموسمين الزراعيين لعامي (٢٠١٨-٢٠١٩) عامل المكافحة الحيوية (التريكودرما هرزيانم) تم عزله في معمل كلية الزراعة سابا باشا جامعة الإسكندرية ، مصر . في الاختبار المعملي تم تقدير تأثير المعاملات الحيوية على نمو الفطر الممرض. في تجربة الصوبة تم إضافة المعاملات بثلاث طرق إضافة : الرش و حقن التربة و خليط بين الطريقتين. في التجربة المعملية المعاملات (الشيتوزان عند تركيز ٤ جم / لتر يليه التريكودرما ثم مركب التوب برفكت عند تركيز ٤ مل / لتر) قامت بتثبيط نمو الفطر الممرض (الفيوزاريوم اكسيسبوريم) بمعدل ٨٦.٣% ، ٨٥.٩% ، ٧٨.٢% على الترتيب. في تجربة الصوبة النتائج اظهرت أن الخلط بين طريقتين الإضافة (الرش الورقي والحقن في التربة) لها دور مهم في مكافحة مرض الذبول الفيوزارمي ، بالإضافة إلى أن زيت الزعتر اعطى اقل فاعلية في مكافحة المسبب المرضي . اعلى تقدير للكلوروفيل كان ٤٨.٢٣% نتيجة المعاملة بالتريكودرما يليها المعاملة بالشيتوزان ٤٧.٦٦% ثم المعاملة بمركب توب برفكت ٤٤.٩٧% دون وجود فرق معنوي بين طريقة الخلط بين الرش و الحقن لمعاملتي التريكودرما و الشيتوزان ، علاوة على ذلك المعاملة بالشيتوزان و التريكودرما و التوب بيرفكت اظهرت زيادة معناويه في الوزن الجاف و الوزن الرطب لنباتات الفراولة و أقل نسبة إصابة و أقل شدة إصابة بالمرض ، كذلك أظهرت هذه المعاملات أعلى طول للنباتات مقارنة بالنباتات الغير معاملة.