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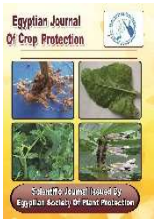

Inhibitory effect of some plant extracts and *Trichoderma* spp. on growth of *Fusarium oxysporum* causing wilt of pepper (*Capsicum annuum* L.)

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ABSTRACT

Fusarium wilt, a disease induced by *Fusarium oxysporum* f.sp. *capsic*i, has a substantial impact on the growth of peppers in the Menoufia governorate area of Egypt. Lost production is the consequence of this illness. The efficacy of four aqueous plant extracts was evaluated in greenhouse and laboratory environments to manage the Fusarium wilt pathogen.The extract of *Laurus nobilis, Dianthus caryophyllus, Cinnamomum verum,* and *Azadirachta indica* demonstrated a significant reduction in the growth of mycelia in the *F. oxysporum* isolate in *in vitro* trials.Carnation aqueous extract were the most effective one against Fusarium. The data obtained indicated that the pathogenic fungal growth was completely suppressed at concentrations of 2.5, 5, and 10% of *Dianthus caryophyllus* extract. *Trichoderma harizianum* inhibited the mycelia growth by 93.38% ,followed by *T. Viride* with 89.27%. InThe most effective treatment against Fusarium was the carnation aqueous extract.The data obtained indicated that the pathogenic fungal growth was completely suppressed at concentrations of 2.5, 5, and 10% of *Dianthus caryophyllus* extract. *Trichoderma harizianum* inhibited the proliferation of mycelia by 93.38%, while *T. viride* did so by 89.27%.Compared to the infected plants (positive control), all treatments were efficacious in reducing the incidence of wilt (post-emergence damping off and severity of infection) and increasing certain vegetative growth parameters of pepper plants (plant height and root weight) in spots experiments. The ultrastructural investigations of pepper plants that were treated with *Dianthus caryophyllus* extract revealed an increase in the size of plaside cells, spony cells, in comparison to the infected leaves, as well as the quantity of xylem arms and phloem layers.

Key words: Pepper, .*F. oxysporum* f.sp. *capsici* , Plant extracts, *Trichoderma* spp, Light microscopy.

INTRODUCTION:

Throughout the globe, including Egypt, the pepper plant has become an essential vegetable. It is cultivated in open fields and greenhouses at various times of the year (Olatunji and Afolayan, 2018).Pepper possesses a high level of intelligence. The pepper plant has become an indispensable vegetable in numerous regions of the world, including Egypt. It is cultivated in greenhouses and open fields at different periods of the year. While hot peppers contain the digestive stimulant capsaicin, they are high in vitamin A and C, as well as

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a significant quantity of phosphorus, calcium, and iron, and have a high nutritional value. (Pundir, *et al*., 2016).The body is protected from infections and the prevalence of cardiac disease is reduced by the abundance of flavonoids, lutein, carotenoids, and cryptoxanthin (Azlan, *et al*., 2022).

The production of peppers has been diminished as a result of a variety of biotic and abiotic factors, particularly fungal disease(Hussain and Abid, 2011). The most destructive losses in pepper arecaused by fungal infections, including root rot, wilt, damping off, leaf spot, powdery mildew, downy mildew, blight, and anthracnose diseases. These diseases are caused by fungi. (Attia *et al*., 2016). One of the destructive diseases of pepper that can result in successful disease development shortly after its interaction with the organisms is *Fusarium oxysporum*.Upon colonization, the pathogen is entirely dependent on the host for the development of its mycelium and nutrition, resulting in a significant disruption of physiological functions. The fungus can directly penetrate the host through hyphae and may also be aided by the production of specialized enzymes (Muhammad *et al*., 2023). Therefore, it is crucial to manage *F.oxysporum* due to ecological constraints that are associated with the use of synthetic chemicals. However, these chemicals have a detrimental impact on the environment and disrupt biodiversity (Maurya *et al*., 2019).

A cost-effective, environmentally safe, and durable alternative to chemicals is biological control, which is used to mitigate the adverse effects of pathogenic parasites.The development of these parasites, particularly those found in soil, is mitigated through the utilization of plant extracts and microorganisms(Jaywant *et al*., 2017 and Abdel-Aziz *et al*., 2023).By effectively managing Fusarium wilt disease and functioning as an alternative to the use of fungicides, the plant compounds exhibit antifungal activity against a wide range of fungal diseases(Enespa *et al*., 2014).

For an extended period of time, plant extracts have been employed to combat fungal diseases. (Nasrin *et al*., 2018).These extracts are environmentally benign. Neem and willow extracts were employed to combat Fusarium wilt on tomatoes, and the results were that the disease was reduced in its spread(Ali *et al*., 2013). According to Belabid *et al*., 2010), extracts from chili pepper and carnation plants influence mycelial growth of *F. oxysporum*.

Researchers are employing Plant Growth Promoter Fungi (PGPF) to enhance plant defense and immunity, as well as to facilitate in addition to serving as a biofertilizer agent, microorganisms isolated from typical plant rhizospheres are beneficial in their ability toabsorption of minerals from the soil. combat pathogens. mineral absorption from the soil. (Badawy*et al*., 2021). In addition to serving as a biofertilizer agent, microorganisms isolated from typical plant rhizospheres are beneficial in their ability to combat pathogens(Abdelaziz *et al*., 2023).The production of toxic compounds, such as siderophores, by fungi is one of the numerous potential pathways to fugal soil-borne disorders that can be accessed through the use of PGPR(Daigham *et al.,* 2023). Considering all the facts, present study have been undertaken to develop a sustainable bio control strategy using different plant extracts and antagonistic organisms against *Fusarium oxysporium* and determining their relative efficiency.

MATERIAL AND METHODS:

Isolation and identification of the pathogen:

Samples of pepper plants showed symptoms of wilt were collected from different pepper fields in Menoufia governorate, Egypt during the season 2023-2024. The pathogen was isolated by removing the roots and 0.5–1 cm sections of the stem, cleaning them with tap water, sterilizing them with 70% ethanol for two minutes, and rinsing them with sterile water. In a petri dish, four sections of potato dextrose agar (PDA) were arranged.At 25± 2°C, the plate was incubated. The isolates were purified after three days by transferring the hyphal ends of the grown fungi one at a time to fresh PDA plates. After that, they were identified according to the morphological and microscopical characteristics that were specified by (Ammar,2003).

Preparation of extract of the plant parts:

Powders from four plant samples (Table1) were utilized in this investigation. The powders were created by weighing 100 gm of powder from each plant material, mixing it thoroughly with 900 ml of distilled water, and then autoclaving it with steam under pressure at 90°C for 30 minutes (Metwally *etal*., 2002). Each plant extract was implemented at three distinct concentrations:2,5, and 10%. Refrigerated in dark glass receptacles, the aqueous extracts were stored for future research. The bioactivity of plant extracts was assessed using the agar dilution method after the were synthesized(Akaeze and Modupe 2017).

Table 1. Plant materials used in aqueous extracts to bioassay against *Fusarium oxysporum* f.sp.*capsici* .

Botanicals			
Common name	**Scientific name**	**Family**	**Used part**
1-Bay leaf	*Laurus nobilis*	Lauraceae	Leaves
2-Carnation	*Dianthus caryophyllus*	Caryophyllaceae	Flower buds
3-Cinnamon	*Cinnamomum verum*	Lauraceae	Bark
4-Neem	*Azadirachta indica*	Meliaceae	Leaves

Three replicated plates for each treatment were maintained and the results were recorded when the control plate was full with fungal growth. The percent inhibition of the mycelial growth of the pathogen over control was calculated by using the formula given by (Shivapratap*et al*. 1996) as depicted below.

$$PI = \left[\frac{C - T}{C}\right] \mathbf{x\ 100}$$

Wherever, PI: is the inhibitory percentage over control, C: is mycelial radial growth in control plate, T: is mycelial radial growth in treatment.

Isolation and identification of *Tricoderma* spp.

Soil samples from the same farms were used to isolate the microorganisms in question using the rhizosphere soil. Using PDA media, the dilution plate and warcup soil plate procedures were performed. Once this was completed, the dishes were incubated at 25°C for seven days and monitored on a daily basis (Ammar 2003). The color and texture of the colony surface were used to identify the PGPF isolates in

accordance with their morphological and microscopic characteristics**(**Gerlach and Nirenberg 1982).

Effect of *Trichoderma* spp on fungal growth *invitro*.

The antagonistic ability of two tested isolates of *Trichoderma* spp was assessed against *F.o.* f.sp. *capsici* according to the method described by (Gams and Bissett, 2002). Three days old cultures *of T. harzianum* and *T. viride* were used as sources of antagonistic inocula. A disc of each one of the tested *Trichoderma* isolates (4mm) was placed 20 mm away from the edge of the PDA plates (9 cm). A pathogen disc was placed in the opposite edge of the Petri plate (Devi *et al*., 2015). Three replicated plates for each treatment were incubated at 25^{o}C until the growth of the control plate completely covered the check plates.

Antagonism test of plant extracts and *Trichoderma* spp against *F. o. capsici invivo.*

Soil sterilization and infestation:

Each of the isolated pathogen and Trichoderma spp was cultivated in receptacles on sterilized barley medium (75 g barley grains + 25 g sand + 100 mL water). In order to sterilize the plastic containers (15 cm in diameter) employed in this experiment, they were immersed in 5% formalin for 5 minutes and subsequently retained for a week until the formalin evaporated. After being sterilized by autoclaving a clay loam soil and sand mixture (2:1) at 121°C for 20 minutes, the soil was contaminated with a pathogen at a rate of 3% of the soil weight. A plastic container weighing one kilogram was subsequently used to confine the soil(Ammar 2003).The colonized soil was irrigated on a daily basis for a week before sowing, thereby allowing the fungus to spread throughout the soil(Ali and Bushra, 2023).After that, the roots of pepper seedlings (Cv. balady) were immersed in the plant extracts at a concentration of 10% for a period of 30 minutes. The medicated seedlings were transplanted into soil that was infested(Seema and Devaki, 2010). Negative control (N.C.) was a pepper seedling that was not treated and was placed in sterilized soil. The positive control (P.C.) was established by planting untreated pepper seedlings in soil that was exclusively contaminated with *F. oxysporum*. In the second experiment, sterilized soil was infested with *F. oxysporum* f.sp.*capsici* and *Trichoderma* spp at a rate of 3% of soil weight.The fungus was allowed to disseminate throughout the soil by irrigating the containers daily in a greenhouse at a temperature of 24±2 °C. The post-emergence damping-off (%) was calculated by dividing the total number of emerged seedlings by the number of plants exhibiting disease indicators seven days after transplanting. Plant growth parameters, including disease severity, fresh root weight, and plant height, were assessed over a sixty-day period following transplantation.According to the following formula equation,a plant's death is indicated by a score of 5, while a score of 4 indicates pronounced wilting and necrosis, a score of 3 indicates slight wilting and necrosis, a score of 2 indicates slight wilting with pronounced chlorosis, a score of 1 indicates chlorosis of lower leaves, and a score of 0 indicates no symptoms:

$$\textbf{Disease severityDS}(\%) = \left[\frac{\boldsymbol{n}\ \mathbf{x}\ \mathbf{v}}{\boldsymbol{N}\ \mathbf{x}\ \mathbf{V}}\right] \mathbf{x}\ \mathbf{100}$$

Where: n = degree of infection rated on a scale of 1-5, v = number of plants in a category, N = highest degree of infection rate, and V = total number of plants screened (EPPO, 1997).

Light microscopy:

The light microscope was used to analyze semi-thin sections of healthy, infected (without any treatments), and treated infected leaves with *Dianthus caryophyllus* extract (the most effective treatment) after contrast staining, as previously describedby Ruzin (1999).The research was conducted at the LM facility of the Faculty of Agriculture Research Park at Cairo University.

Statically analysis:

The obtained data were subjected to analysis of variance (ANOVA) using Costat software). Duncan multiple range tests (DMRT) at $p < 0.05$ level was used for means separation (Gomez and Gomez 1984).

RESULTS:

***In vitro*: Evaluation of plant extracts against *F.o. capsici*:**

At concentrations of 2.5, 5, and 10%, the antifungal activity of four plant extracts was evaluated against *F. oxysporum*.Data present in Table (2) and Fig (1)indicate that In all experimental assays, the fungal growth of *F. oxysporum* was effectively inhibited by all concentrations of plant extract that were tested. It was obvious that increasing the concentration of tested extracts reduced fungal growth more effectively. Carnation extract was most effective in inhibiting the growth of fungal growth than the other plant extracts (100 %) at three concentrations, followed by Cinnamon extract (98.01 %), Neem extract (92.82%) and Bay leaf extract (88.12 %) at 10%, respectively.

Antagonism of *Trichoderma* spp isolates against *F .oxysporum*f. sp. *capsici.*

Trichoderma harzianum and *T. viride* were isolated from healthy pepper rhizosphere were tested as biocontrol agents. The results in Table (3) and Fig.(2) clearly demonstrate that all tested *Trichoderma spp* inhibited *F. oxysporum* mycelium growth. The growth inhibition of Fusarium was (93.38 %) by *T. harzianum* while it was (89.27 %) by *T.viride.*

Table (2):Efficacy of different concentration of aqueous plant extracts on the growth of *F. o.capsici in vitro* assay:

Plant extracts	Concentration (%)	Linear growth (mm)	Growth reduction* (%)
A- Bay leaf	2.5	28.15^{b}	67.55^{g}
	5	15.55^{c}	82.05^{f}
	10	10.3^{e}	88.12^{d}
B- Carnation	2.5	00.00^{h}	100.00^{a}
	5	00.00^{h}	100.00^{a}
	10	00.00^{h}	100.00^{a}
C- Neem	2.5	15.55^{c}	82.80^{f}
	5	12.5^{d}	85.80^{e}
	10	6.23^{f}	92.82^{c}
D- Cinnamon	2.5	5.23^{f}	93.97^{c}
	5	2.50^{g}	97.11^{b}
	10	1.73^{g}	98.01^{b}
Control	-	86.77^{a}	00.00^{h}
L. S. D. 0.05	-	1.49	1.45

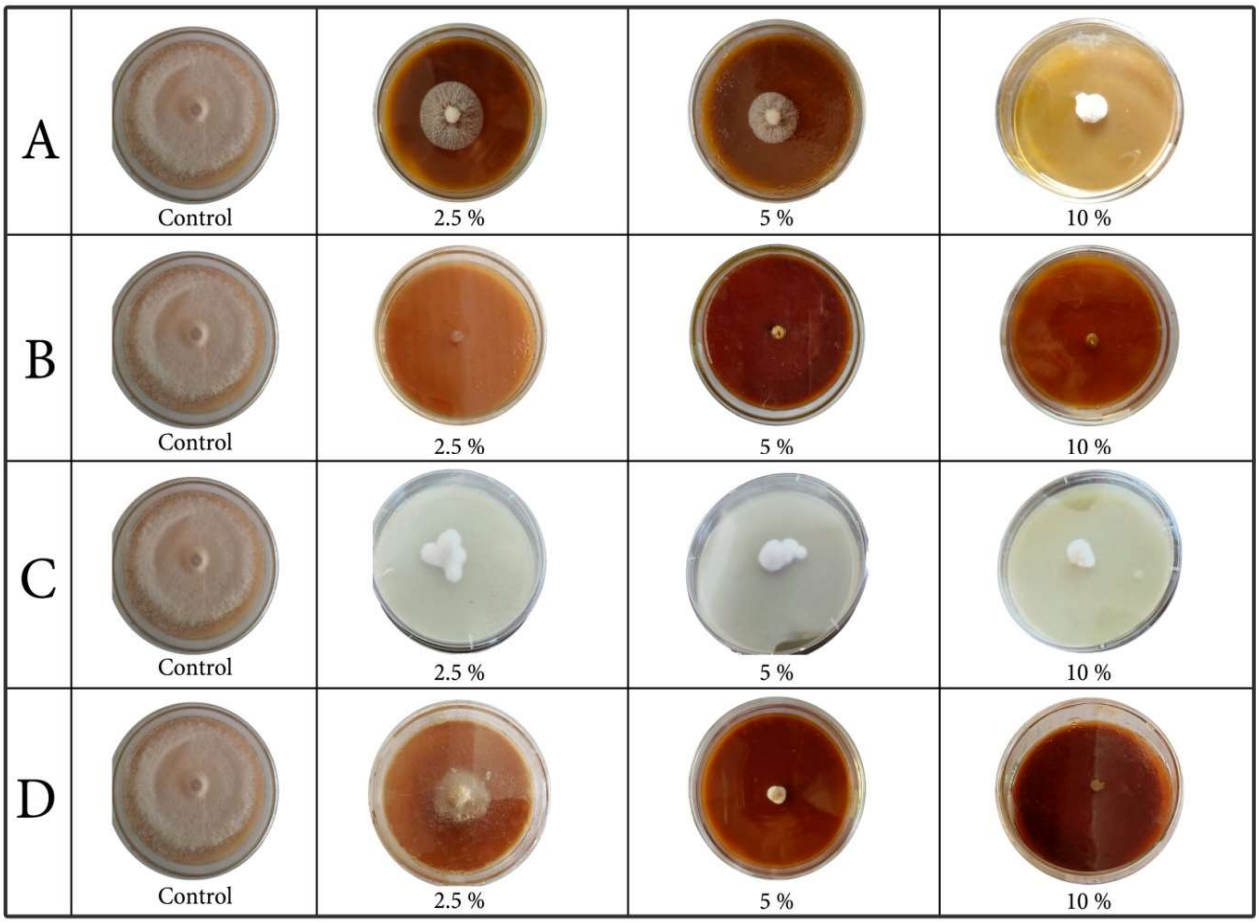


Fig (1): Inhibitory effect of different concentrations of teseted plant aqueous extracts on the growth of *F. oxysporum* grown on PDA medium, A:Bay leaf treatment B: Carnation treatment C: Neem treatment and D: Cinnamon treatment.

Table (3): Effects of *Trichoderma* spp on the mycelia growth of *F. oxysporum* using*invitro* assay.

Treatments	Growth of mycelium (mm)	Growth inhibition (%)	Mode of action	
			O. G *(mm)	I.Z**(mm)
T. harzianum	5.33^{c}	93.38^{a}	+	-
T. viride	9.31^{b}	89.27^{b}	+	-
Control	86.77^{a}	0.00^{c}	-	-
L.S.D 0.05	2.61	2.26	-	-

*O.G: Overgrowth (mm) **I.Z: Inhibition zone (mm)

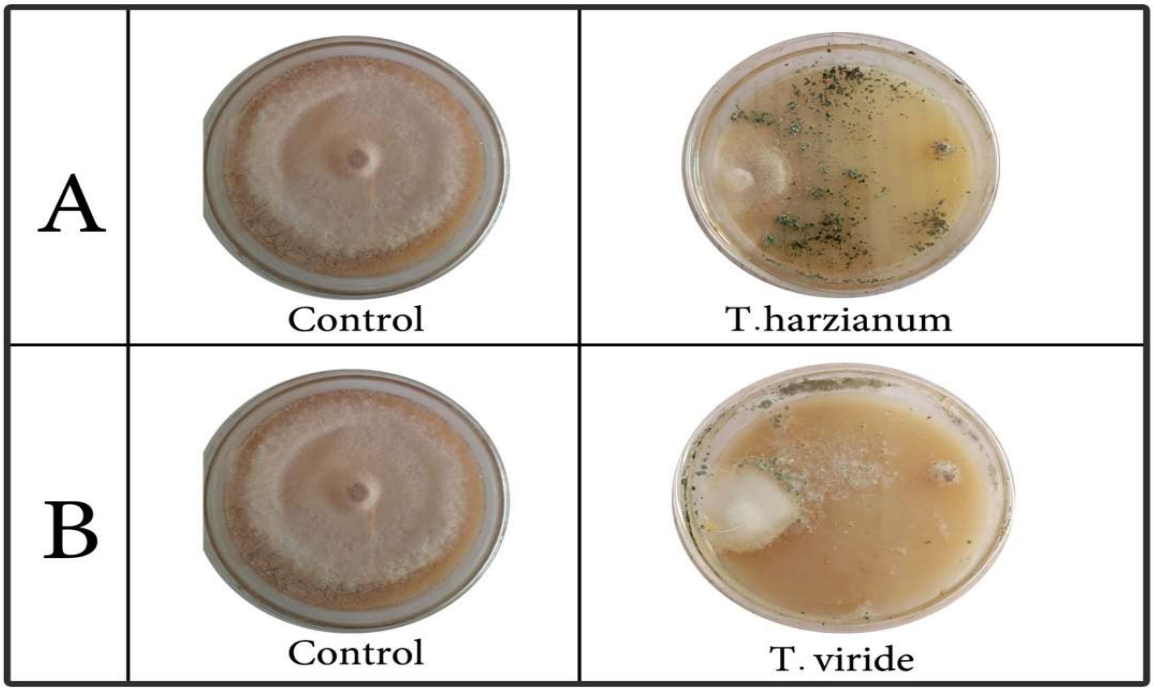


Fig (2):Effect of tested *Trichodermaspp* on the growth of*F.oxysporum* grown on PDA medium, A: *T. harzianum* B:*T. Viride*

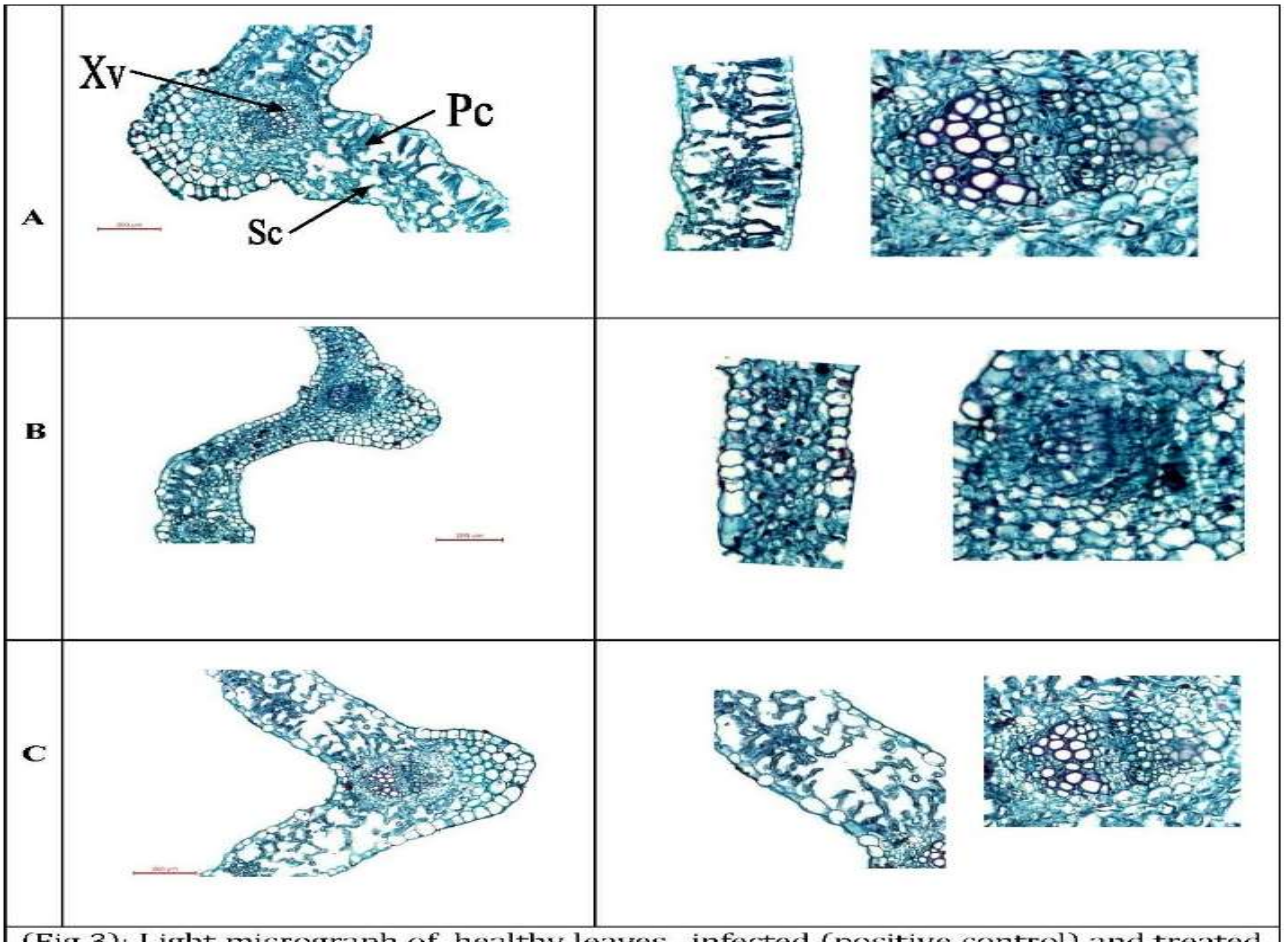
***In vivo*: Evaluation of tested plant extracts against *F. oxysporum*:**

In comparison with the positive control, all tested plant extracts significantly decreased post-damping off pepper seedlings inoculated with *F. oxysporum*. Results in Table (4) showed that carnation extract was the most effective causing decreasing in Post-emergence damping-off, followed by cinnamon extract and neem extract. Data present in Table (3) indicate that all *Trichoderma* spp. isolates recuced post emergence percentage of *F. oxysporum*-inoculated seedlings as compared to pathogen-inoculated and untreated control. The seveerity of Fusarium root rot was evaluated 60 days after transplanting and found to be significantly lower between the treatments tested and the positive control (Table 3). Maximum decrease was achieved by carnation. The data also showed that all tested plant extracts and *Trichoderma* spp significantly increased plant height of treated plants compared to positive control (Table 3), in addition, the fresh root weight mean was significantly increased in carnation treatment (5.11 g) compared to positive control (3.08 g).

Light microscopy:

The investigations revealed large difference between healthy, treated and non-treated tissues. Mesophyll layers (photosynthetic cells) were reduced and compact in infected plants and the size of plaside cells became short and compact. Also spongy cells were unorganized when compared with healthy ones. The number of xylem arms and phloem layers was reduced and disorganized in the infected cells than in healthy ones. Treated plants with *Dianthus caryophyllus* extract increased the size of plaside cells, spony cells and the number of xylem arms and phloem layers than in infected leaves (Fig3).

Table (3): Invivo: Evaluation of tested plant extracts and Trichoderma spp on the disease incidence.				
Measurements / Treatment	Post damping- off (%)	Disease severity (%)	Plant height (cm)	Root weight (g)
Carnation extract	5f	1.0bc	33.86a	5.11a
Bay leaf extract	20c	1.8ab	30.91cd	4.01ab
Neem extract	10e	1.4b	32.60abc	4.25ab
Cinnamon extract	10e	1.3b	33.11ab	4.82ab
T. harizianum	15d	1.4b	32.67abc	4.47ab
T. viride	30b	1.9ab	31.44bcd	4.11ab
Negative control	0.00g	0.0c	34.13a	5.67a
Positive control	60a	2.7a	29.68d	3.08b
LSD0.05	0.77	1.06	1.81	1.81



(Fig 3): Light micrograph of healthy leaves , infected (positive control) and treated pepper leaves with *Dianthus caryophyllus*, showing different changes in cells and tissues (200X).**A:** healthy leaves **B**: infected (positive) **C**: pepper leaves treated with *Dianthus caryophyllus* extract.

DISCUSSION:

Peper is susceptible to *Fusarium oxysporum* f.sp. *capsici*, a disease that is both harmful and widespread(Jaywant, *et al.,* 2017).Scientists focused on reducing the danger of plant diseases by using biotic and abiotic inducers to stimulateplant physiological immunity and pathogen resistance (Eastburn *et al*., 2011 and Elbasuney*et al.*, 2022). Strong and unambiguous evidence of disease resistance is the reduction of disease symptoms and the severity of infection.In the current study, the fungitoxic character of four botanicals/plant extracts was assessed in vitro and in vivo against F. oxysporum at concentrations of 2.5, 5, and 10%. The radial growth was significantly inhibited by the aqueous emulsions of the extracts at all concentrations. Carnation extract substantially reduced mycelial growth by up to 100% at three concentrations, while bay leaf extract exhibited the least growth (88%) among the botanicals.Plant extracts have been employed in numerous investigations to combat *F. oxysporum*. For instance, Obongoya *et al.,* (2009) discovered that cinnamon extracts completely inhibited the radial growth of *Fusarium oxysporum* mycelium *in vitro* . According to Belabid *et al*. (2010) and Sidawi *et al.* (2010), Carnation and Chili Pepper plant extracts affect the mycelial growth of *F. oxysporum*. The leaf extracts of neem (*Azadirachta indica*) have been reported as highly toxic to *Fusarium oxysporum* showing complete inhibition of mycelial growth and spore germination (Ramaiah and Raj Kumar, 2015).

Fungal species from the genus *Trichoderma* are ubiquitous and can be effortlessly extracted from soil. Numerous researchers have documented the utilization of *Trichoderma* species as biocontrol agents against a variety of plant diseases(Coley-Smith, *et al*., 1991).The current study evaluated two *Trichoderma* isolates against FOL. A relatively higher level of FOL inhibition was demonstrated by *Trichoderma harzianum* than by *T. viride*. In addition, other investigations have demonstrated the antagonistic impact of *Trichoderma* spp., particularly *T. harzianum, T. viridae*, and *T. hamatum*, and have effectively managed *F.oxysporum* f. sp. *persici* on pepper(Hewedy *et al*.,2020;Ali and Bushra, 2023; Amer *et al*., 2023 and Ziaul *et al*., 2023).

The indirect exercise of biocontrol by *Trichoderma* can be facilitated by three mechanisms: direct parasitism on pathogens(Yedidia,*etal*.,1999), competition for nutrients and space, and the production of volatile or diffusible metabolites and antibiotic metabolites (Chet, *et al*., 1997).However, mycoparasitism is regarded as a direct biocontrol mechanism and may also have a stimulatory effect on plant growth (Naseby, *et al*., 2000).

In pot experiments, *FOL*-inoculated pepper plants caused increasing in post damping off, disease severity. However soil treatments with plant extracts and *Trichoderma* spp reduced harmful effects of pathogenic fungus and thus enhanced pepper plant growth and yield. Carnation extracts was the most effective treatments against FOL. *T. harzianum* was shown to be the most virulent isolate in antagonizing *FOL,* the efficacy of this isolate was better than *T. viridae* . Other studies revealed the antagonistic effect of *Trichomera spp* especially *T. harzianum* successfully controlled *F. oxysporum* f. sp *capsici* on pepper(Akrami and Yousef , 2015 , Amer *et al*., 2023 and Ziaul *et al*., 2023).

The study of histological abnormalities continues to be influenced by light microscopyin that it allows for the examination of a wider variety of tissue to determine the presence or absence of inclusion bodies(Matthews, 1991).A significant increase in cells and tissues compared to infected ones was observed after the application of inducers (*Dianthus caryophyllus*) similar results obtained by Sayed *et al*., (2001).

CONCLUSIONS:

Biocontrol is the best and effective control strategy when dealing with the soil borne pathogens such as *Fusarium* species. This method has many advantages such as being ecofriendly, cost effectiveness and extended plant protection. All tested plant extracts and *Trichoderma* spp were effective to control Fusarium wilt. However, extracts of carnation gave the best results.

Authors Contributions

Conceptualization, data curation, formal analysis, Investigations, Methodology, writing original drafts, and writing and editing; read and agreed to the published version of the manuscript.

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The data presented in this study are available on request from the corresponding author.

Conflicts of interest:

The author declares no conflict of interest.

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