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# Bio-efficacy of some Botanicals, Antagonistic fungi, and Fungicides against *Fusarium oxysporum* f.sp. *phaseoli* causing Fusarium wilt on common bean plants

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## ABSTRACT

Wilt of common beans (*Phaseolus vulgaris* L.) caused by *Fusarium oxysporum* f.sp. *phaseoli*, has been found to be important in Menoufia governorate, Egypt. The objective of this study was to evaluate the efficacy of five plant extracts, three *Trichoderma* isolates, and two systemic fungicides in controlling Fusarium wilt infestations under laboratory and greenhouse conditions. In vitro tests revealed that all of the plant extracts tested exhibited fungicidal activity against *F.oxysporum* f.sp. *phaseoli*, albeit to varying degrees of efficacy. *Cuminum cyminum* and *Dianthus caryophyllus* aqueous extracts were the most effective against Fusarium. All three *Trichoderma* isolates inhibited the mycelium growth of the pathogen. Furthermore, the efficacy of two systemic fungicides (Rizolex-T and Topsin M) were investigated and both tested fungicides reported a significant reduction in the growth of the pathogen compared to the control. *In vivo* evaluation the results showed that all tested agents were effective in reducing wilt incidence (pre-and post-emergence, survival of plants, and severity of infection) and increasing some vegetative growth parameters of bean plants (plant height, number of branches, and number of leaves/plants) compared to the infected control. In comparison to synthetic fungicides, our study demonstrated that the use of aqueous plant extracts could be an applicable, safe, and cost-effective method for controlling *F. oxysporum* f.sp. *phaseoli*. In addition, our study found that local isolates of *T.harzianum*, *T. asperellum*, and *T.viride* have the potential to be used as biological control agents against *F. oxysporum* f.sp. *phaseoli*.

**Key words:** Common bean. *F. oxysporum* f.sp. *phaseoli*. Plant extracts. *Trichoderma* spp. Rizolex-T.

## INTRODUCTION

The common bean (*Phaseolus vulgaris* L) is among the most globally important legume crops. It is a major source of dietary fiber, calories, proteins, minerals, and vitamins for millions of people worldwide (Nemli *et al*., 2015). In Egypt, it is considered the chief vegetative. According to the report of Food and Agriculture Organization (FAO, 2020), the total cultivated area of dry beans in Egypt reached 36719 ha which yielded 144809 tons, with an average of 39437 hg/ha.

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Common beans are affected by various bacterial, fungal and viral diseases, which in turn produce a heavy loss to the crop (Buruchara and Camacho 2000). Fusarium wilt, caused by a soil-borne pathogenic fungus (*Fusarium oxysporum* f.sp. *phaseoli*) (FOP), is one of the most serious diseases of common beans, causing 50–100% of crop losses due to early wilting. It is found in many parts of the world (Schwartz *et al*., 2005). This fungus causes vascular wilt over the roots and concludes the cortex with the stele. The vascular tissues of the root, and then the stem, are colonized by the development of the fungal hyphae and the

movement of conidia in the transpiration stream (Tjamos and Bechman 1989). Initial symptoms appear as chlorosis and distortion of the lower leaves, often on one side of the plant. Foliar yellowing, necrosis, and plant stunting become more pronounced as the disease develops. Wilting happens on the diseased side of the plant, followed by vascular discoloration and stem necrosis. The whole plant wilts and dies as the pathogen moves into the stem (Carvalho *et al*., 2014).

Control of Fusarium vascular wilts in susceptible cultivars is very difficult, and there are only a few recommended management options (Sidawi *et al*., 2010). Antimicrobial compounds used to treat Fusarium wilt in common beans, such as fungicides, are generally uneconomical, have a severe environmental impact, and promote the establishment of fungicidal resistance variations. In the last few decades, scientists have been looking for non-chemical approaches to controlling postharvest disease (Abeysinghe 2007). Biological control is considered an environmentally suitable alternative to current chemical treatment methods for controlling soil-borne pathogenic fungi causing wilt (Otadoh *et al*., 2011). Natural products, such as botanical amendments or plant extracts, are being used to control fungal diseases in plants as an alternative to synthetic fungicides due to their fewer negative effects on human and environmental health hazards and implications. This may be used for expressing new, safer, and ecofriendly fungicides (Ramaiah and Garampall 2015).

Although chemical pesticides are sometimes more suitable, biopesticides derived from natural resources are a greater option. Recently, the various botanicals became increasingly prominent in the field of plant protection. Plant-derived (botanical) protectants (PDPs) provide a competitive improvement over synthetic pesticides because they are safer and cheaper (Himanshu *et al*., 2022)

In addition, antagonistic fungi, *Trichoderma* spp., are identified to have biological mycoparasites and which are used commercially by way of biocontrol against a range of soil-borne pathogenic fungi such as, Pythium, Rhizoctonia, Machrophmina and Fusarium strains plus a product of biological notice (Verma *et al*., 2007, Abdel-lateif and Bakr,2018, Khalifa *et al*., 2019, Hewedy *et al*.,2020). Thus, the present work has been designed and undertaken to evaluate the potential of some plant extracts, antagonistic fungi, and fungicides against *Fusarium oxysporum* f.sp. *phaseol* in laboratory and under greenhouse conditions.

## MATERIALS AND METHODS

### Isolation and identification of the causal organisms

Samples of naturally infected bean plants were collected from different locations of Menoufia governorate, Egypt. Samples showing different symptoms ranging from foliar chlorosis, necrosis, stunting, wilting, vascular discoloration, stem necrosis and death were collected. Infected portions were cut into small pieces, washed thoroughly with running water to remove any observing soil particles. These pieces were surface sterilized by immersing them in a 5% sodium hypochlorite solution for 2 min, followed by 70% ethanol for 2 min, then washed several times in sterilized water, and finally dried with sterilized filter papers. Four external sterilized pieces were transferred onto (PDA) media containing streptomycin sulphate to avoid any bacterial contamination. Petri plates were incubated

at 25°C for 4–7 days and observations were recorded (Jens *et al*., 1991). Hyphal-tips of developed fungi were transported individually to new PDA plates, then identified according to their morphological and microscopical characters as defined by (Ammar, (2003). Identification was confirmed by the Botany Department, Faculty of Agriculture, Menoufia University, Shebin-El-Kom, Egypt.

***In vitro* evaluation of tested bioagents and fungicides in controlling *Fusarium oxysporum f.sp. phaseoli***

**Efficacy of plant extracts on the fungal growth**

In this study, aqueous plant extracts were used. Five plant materials involved in this study were collected and identified in (Table 1). Powders of five plant samples were used in this study. 100 gm of each plant material's dry powder was mixed thoroughly with 1000 ml of distilled water before being autoclaved with steam under pressure at 90 °C for 30 minutes (Metwally *et al*. 2002). Three concentrations of each plant extract *i.e*., 2.5, 5 and 10%, with three replicates, were used. The aqueous extracts were kept in dark glass bottles in the refrigerator for further studies. Plant extracts were prepared and evaluated for their bioactivity using the agar dilution method (Akaeze and Modupe, 2017).

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

| Plant Extracts | | | |
|---|---|---|---|
| **Common Name** | **Scientific Name** | **Family** | **Used Part** |
| Carnation | *Dianthus caryophyllus* | Caryophyllaceae | Flower buds |
| Chili Pepper | *Capsicum annum* | Solanaceae | Fruits |
| Cumin | *Cuminum cyminum* | Apiaceae | Seed powder |
| Pomegranata | *Punica granatum* | Lythraceae | Peel |
| Tumeric | *Curcuma longa* | Zingiberaceae | Rhizomes |

**Efficacy of some biocontrol agents on fungal growth**

Healthy common bean plants were collected from the same fields and the rhizosphere soil was used for isolating the associated microorganisms. Warcup soil plate and dilution plate method were conducted using PDA medium. Then the plates were incubated at 25°C for 7 days and examined daily (Ammar 2003). The obtained isolates were primarily identified based on their morphological and physiological characteristics (Gerlach and Nirenberg 1982). The antagonistic ability of three tested isolates of *Trichoderma* spp. was assessed against *F. oxysporum* f.sp. *phaseoli* according to the method described by (Fokkema 1973). Three days old cultures of *T. harzianum*, *T. viride* and *T. asperellum* 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 center 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.

**Efficacy of fungicides on the fungal growth**

In this experiment, the fungitoxicity of two systemic fungicides (Table 2) was evaluated against *F. oxysporum* f.sp. *phaseoli*. Three concentrations, i.e., 50, 100 and 200 ppm (Amal, 2009), were tested individually to assess their effect on pathogen growth inhibition in a petri dish plate. Inoculated plates were incubated for 5-7 days at 25°C and two diameters of every dish were measured daily until the full growth of fungus was noticed in the control. The average diameter and growth reduction were calculated.

**Table 2**. The fungicides used for controlling common bean wilt disease

| Trade name | Active ingredient | Recommended Dose (g a.i.) | Manufacture |
|---|---|---|---|
| Rizolex- T ® 50% Wp | Tolclofos-methyl 20% +Thiram 30% | 3g/1kg seeds | Sumitomo, Japan |
| Topsin M ® 70% Wp | Thiophanate- methyl 70% | 1g/1L | Nippon Soda Co., LTD, Japan |

Three replicated plates for each treatment were maintained and the results were recorded when the control plate was full of fungal growth. The fungitoxicity was carried out in terms of percent mycelial growth inhibition against the tested fungus growth, and was calculated using the following formula:

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

Where, PI: is the percent inhibition over control, C: is mycelial radial growth in control plate, T: is mycelial radial growth in treatment (Shivapratap *et al*. 1996).

**_In vivo_ evaluation of different agents in controlling _F. oxysporum_ f.sp. _phaseoli_**

In this experiment, the antifungal activity of tested agents was evaluated under greenhouse conditions at the farm of the Faculty of Agriculture, Menoufia University, Shebin-El-Kom, Egypt.

**Pots and soil sterilization:**

For greenhouse experiments, common bean seeds (Giza 6) were used. Seeds were superficially sterilized for 2 minutes with 5% sodium hypochloride then rinsed with sterile distilled water and dried at room temperature. The seeds were sown in sterilized plastic pots (15 cm in diameter) containing 1 kilo of clay loam soil and sand (2:1v:v). The soil was heat-treated at 121°C for 20 minutes on two consecutive days, and then cooled before the use. The pots were kept in a greenhouse (24± 2 ºC) and watered frequently until final estimation.

**Inoculum and _Trichoderma_ spp. preparation and soil infestation**

The isolated fungi, *Trichoderma* spp., were individually grown on sterilized barley medium (75 g barley grains + 25 g sand + 100 mL water) in bags, then the bags were incubated for 14 days at 25°C. Sterilized soil was infested with isolate at the rate of 3% of soil weight. The infested soil was irrigated every second day for a week to allow the fungus to spread throughout the soil.

**Cultivation in the infested soil**

The tested plant extracts and fungicides were applied as a seed coating. Common bean (Giza 6) seeds were soaked for 3h in tested plant extract at the concentration (10 %) and then left to air dry before sowing (El-Mougy *et al*., 2007). Comparison treatment included a set of bean seeds coated with fungicides as seed dressing at the recommended dose. Three bean seeds were sown in every pot, and three pots were used as replicates for each treatment. The control treatment had the sterilized soil with the same percentage of sterilized Barley medium. The plants were examined every week for disease incidence determination. The percentage of wilt incidence of beans at pre-and post-emergence stages was calculated after 15 and up to 45 days of the

experimental period, respectively, as following:

$$Pre-emergence\ damping\ off = \frac{Number\ of\ un-emerged\ seedlings}{Number\ of\ sown\ seeds} x100$$

$$Past-emergence\ damping\ off = \frac{Number\ of\ dead\ seedlings}{Number\ of\ sown\ seeds} x\ 100$$

$$Survival\ plants = \frac{Number\ of\ survived\ plants}{Number\ of\ sown\ seeds} x\ 100$$

While the severity of infection (SI) was calculated using 0-4 scale (0= asymptomatic, 1= yellowing, 2= vascular discoloration, 3= wilting, 4= plant dead) according to the following formula equation:

$$Disease\ Index\ (DI)(\%) = \left[\frac{ni\ x\ si}{N\ x\ S}\right] x\ 100$$

Where, ni: number of bean plants with wilt symptoms **si**: value of score of symptoms **N:** total number of tested bean plants S: the highest value of score of symptoms (Cachinero *et al*., 2002).

The average plant height, number of branches, and number of leaves/plant were determined after80 days after seedling.

**Statical analysis**

The data was analyzed using one-way analysis of variance (ANOVA) following by LSD test for mean separation. Statical significance was defined as P value <0.05 (CoStat-statistic software, CoHort software)

## RESULTS

*Fusarium oxysporum* f.sp. *phaseoli* was isolated from naturally infected common bean plants (*Phaseolus vulgaris* L. cv. Giza 6) grown in different locations in Menoufia governorate, Egypt. The diseased plants showed foliar chlorosis, necrosis, stunting, wilting, followed by vascular discoloration, stem necrosis, and dead plants (Fig 1 A, B, C).

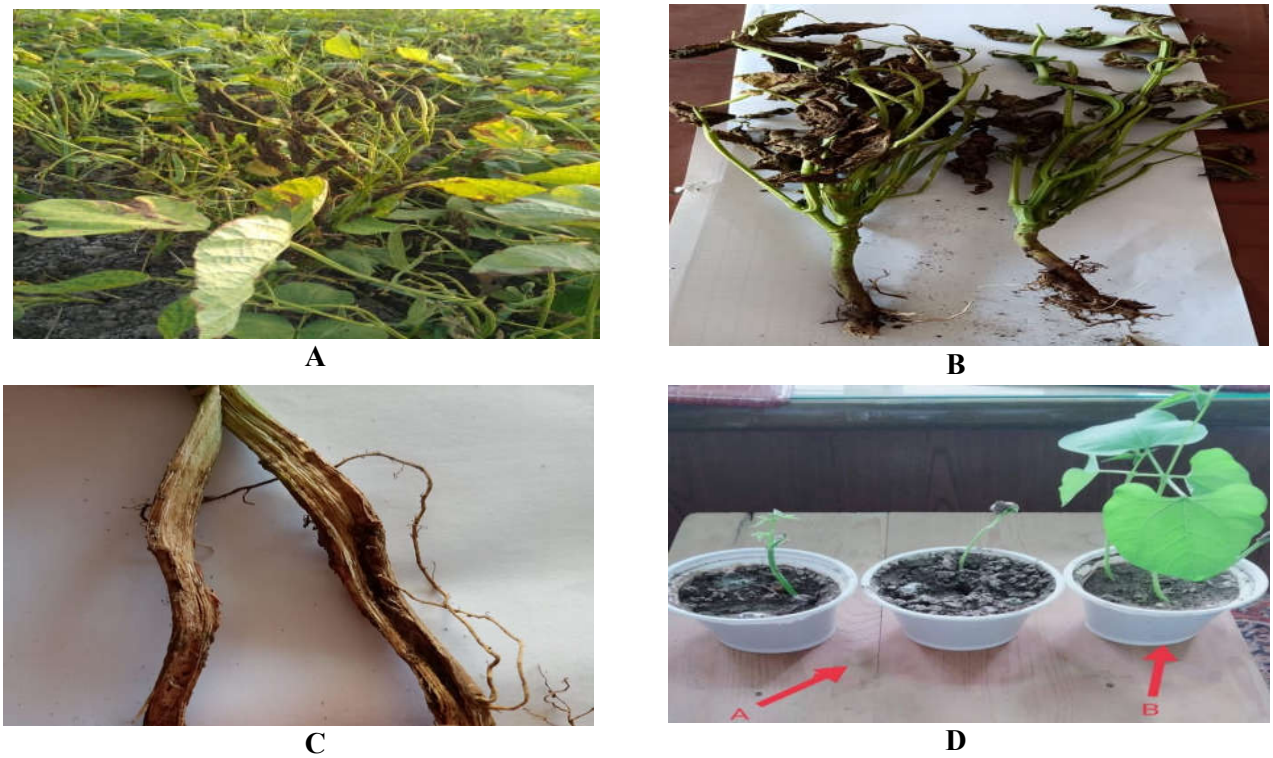


**Fig 1.** A, B: Symptoms of *Fusarium oxysporum* f.sp. *phaseoli* on naturally infected common bean leaves variety (Giza 6), showing wilting and dead plants. C: Vascular discoloration, D: Pre- emergence damping off after seeding, A. infected control, B. healthy control.

***Invitro* evaluation of plant extracts against *F. oxysporum* f. sp. *phaseoli*:**

Results present in Table (3) and Fig (2) indicate that all tested plant extract concentrations were effective in inhibiting fungal growth of *F.oxysporum* f.sp. *phaseoli* in all experimental trials in petri dishes. It was clear that increasing the concentration of any tested extract reduced fungal growth more effectively. Cumin extract was most active in inhibiting the growth of fungal growth than the other plant extracts (94.81 %) at 10 % concentration, followed by Carnation extract (92.82 %), Pomegranate peel extract (82.05%) Chili Pepper extract (73.27 %) and Tumeric extract (65.00 %) respectively.

***Invitro* evaluation of different *Trichoderma* spp. Isolates against *F. oxysporum f. sp. phaseoli***

*T. harzianum*, *T. viride and T. asperellum,* which were isolated from healthy bean rhizosphere, were tested as biocontrol agents. Results shown in Table (4) and Fig. (3) clearly show that all tested *Trichoderma* spp., inhibited the linear growth of *F.oxysporum.* In this request, *T. harzianum* and *T. asperellum* showed the best actions in reducing growth compared to control (71.07 and 67.34 %), respectively. While *T.viride* gave the least efficiency in reducing growth (53.28%). The Inhibition zone recorded 22.91 between *Fusarium* on one side and *T.viride* on the other side.

**Table 3.** Efficacy of different concentration of aqueous plant extracts on the growth of *F. oxysporum* f.sp. *phaseoli in vitro* assay

| Plant extracts | Concentration (%) | Linear growth (mm) | Growth reduction* (%) |
|---|---|---|---|
| Chili Pepper | 2.5 | 32.51$^{e}$ | 62.52$^{h}$ |
| | 5 | 30.33$^{f}$ | 65.03$^{g}$ |
| | 10 | 22.32$^{h}$ | 73.12$^{e}$ |
| Tumeric | 2.5 | 52.41$^{b}$ | 39.48$^{k}$ |
| | 5 | 40.66$^{c}$ | 53.13$^{j}$ |
| | 10 | 30.00$^{f}$ | 65.42$^{g}$ |
| Carnation | 2.5 | 28.15$^{d}$ | 67.55$^{i}$ |
| | 5 | 12.82$^{g}$ | 85.22$^{f}$ |
| | 10 | 6.231$^{k}$ | 92.82$^{b}$ |
| Cumin | 2.5 | 12.31$^{j}$ | 85.80$^{c}$ |
| | 5 | 7.11$^{jk}$ | 91.80$^{bc}$ |
| | 10 | 4.50$^{i}$ | 94.81$^{a}$ |
| Pomegranate peel | 2.5 | 32.48$^{e}$ | 62.56$^{h}$ |
| | 5 | 24.32$^{h}$ | 72.35$^{e}$ |
| | 10 | 15.55$^{i}$ | 82.05$^{d}$ |
| Control | - | 86.77$^{a}$ | 00.00 |
| L. S. D. 0.05 | - | 1.09 | 1.03 |

Means within the same column followed by the same letter(s) are not significantly different (P≥ 0.05, LSD test)*: Growth reduction (%) is calculated relative to the control [control – treated / control x 100].

**Table 4.** Efficacy of different *Trichoderma* spp. isolates on the growth of *Fusarium oxysporum* f.sp. *phaseoli in vitro* assay

| *Trichoderma* spp. | Linear growth (mm) | Growth reduction (%) | Mode of action | |
|---|---|---|---|---|
| | | | O. G* | I.Z**(mm) |
| *T. harizianum* | 25.10 $^{d}$ | 71.07$^{a}$ | + | - |
| *T. viride* | 40.53 $^{c}$ | 53.28 $^{b}$ | - | 22.91 |
| *T. asperellum* | 28.33$^{b}$ | 67.34$^{c}$ | + | - |
| Control | 86.77 $^{a}$ | - | - | |
| L. S. D. 0.05 | 1.23 | 1.08 | - | |

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

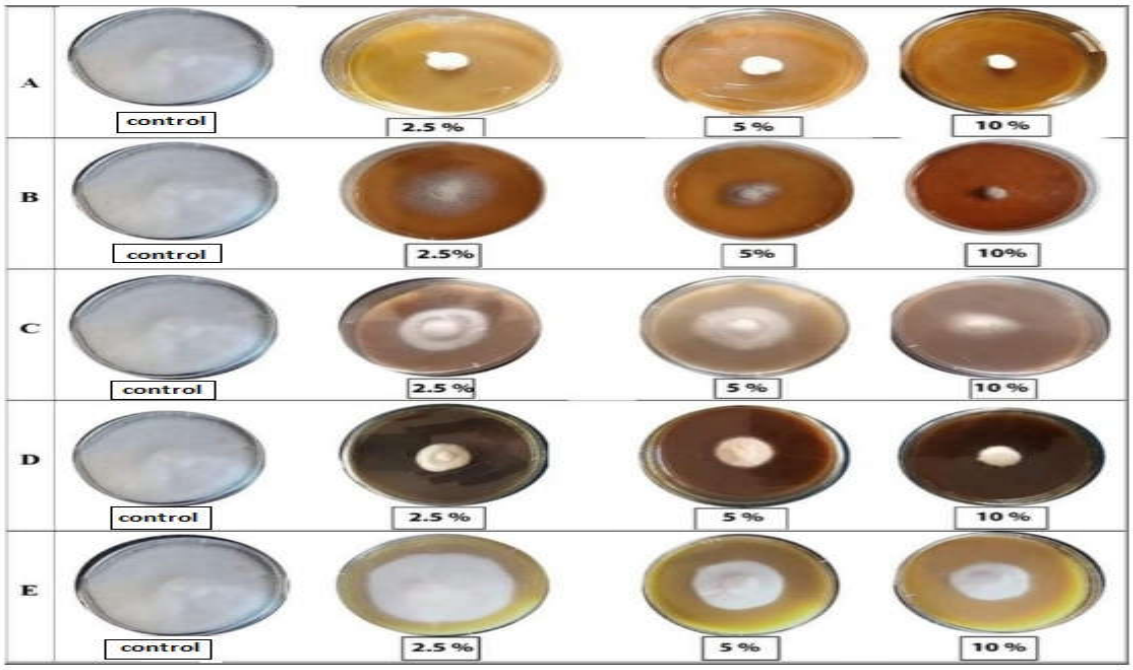


**Fig 2.** Inhibitory effect of different concentrations of selected plant water extracts on the growth of *F. oxysporum* f.sp. *phaseoli* grown on PDA medium, **A**: and Cumin treatment **B**: Carnation treatment **C**: Chili Pepper treatment **D**: Pomegranate peel treatment **E**: Tumeric treatment.

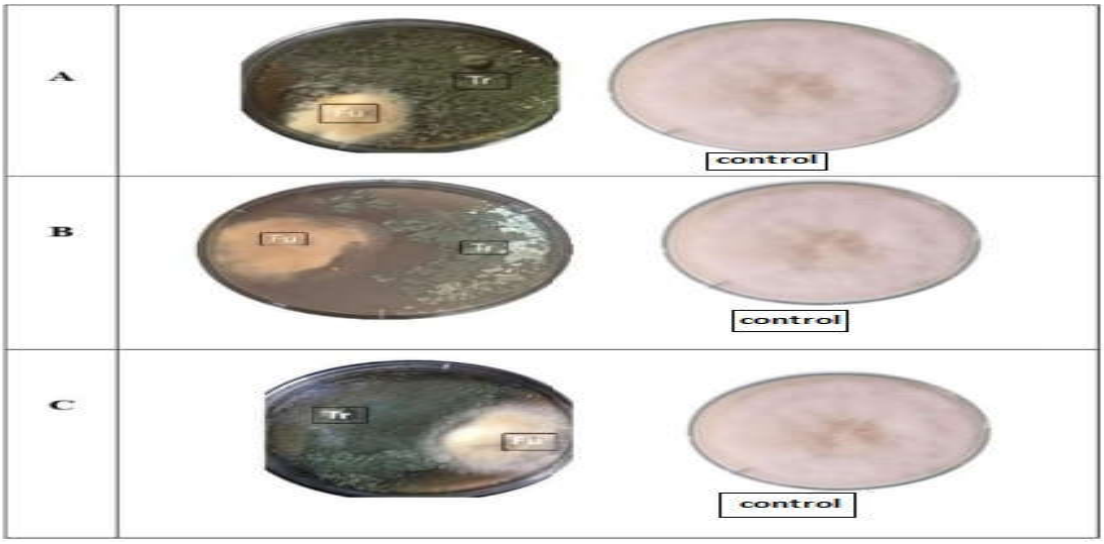


**Fig 3.** Effect of tested *Trichoderma* spp. isolates on the growth of *F.oxysporum* f.sp. *phaseoli* grown on PDA medium, **A**: *T. harizianum* treatment **B:** *T. viride* treatment **C**: *T. asperellum* treatment .

### *In vitro* evaluation of selected fungicides against *F. oxysporum* f. sp. *Phaseoli*

Results presented in Table (5) and Fig (4) indicate that both tested fungicides at all concentrations (50, 100 and 200 ppm) reduced the growth of *F.oxysporum* than control. Increasing the concentration of both fungicides caused a significant reduction in fungal growth. The highest effect on growth of *F.oxysporum* f.sp. *phaseoli* was achieved by Rhizolex- T ( 97.27 % at 200 ppm ) followed by Topsin-M ( 84.60 % at 200 ppm ).

**Table 5.** Effect of different concentrations of two fungicides on the growth of *Fusarium oxysporum* f. sp. *phaseoli in vitro*

| Fungicide | Concentration (ppm) | Linear growth (mm) | Growth reduction (%) |
|---|---|---|---|
| Rhizolex- T | 50 | 14.98[d] | 82.72[d] |
| | 100 | 8.26[f] | 90.47[b] |
| | 200 | 2.03[g] | 97.65[a] |
| Topsin-M | 50 | 23.07[b] | 72.24[f] |
| | 100 | 20.47[c] | 76.39[e] |
| | 200 | 13.35[e] | 84.60[c] |
| Control | - | 86.77[a] | 00.00 |
| L. S. D. 0.05 | - | 0.804 | 0.654 |

Means within the same column followed by the same letter(s) are not significantly different (P≥ 0.05, LSD test)

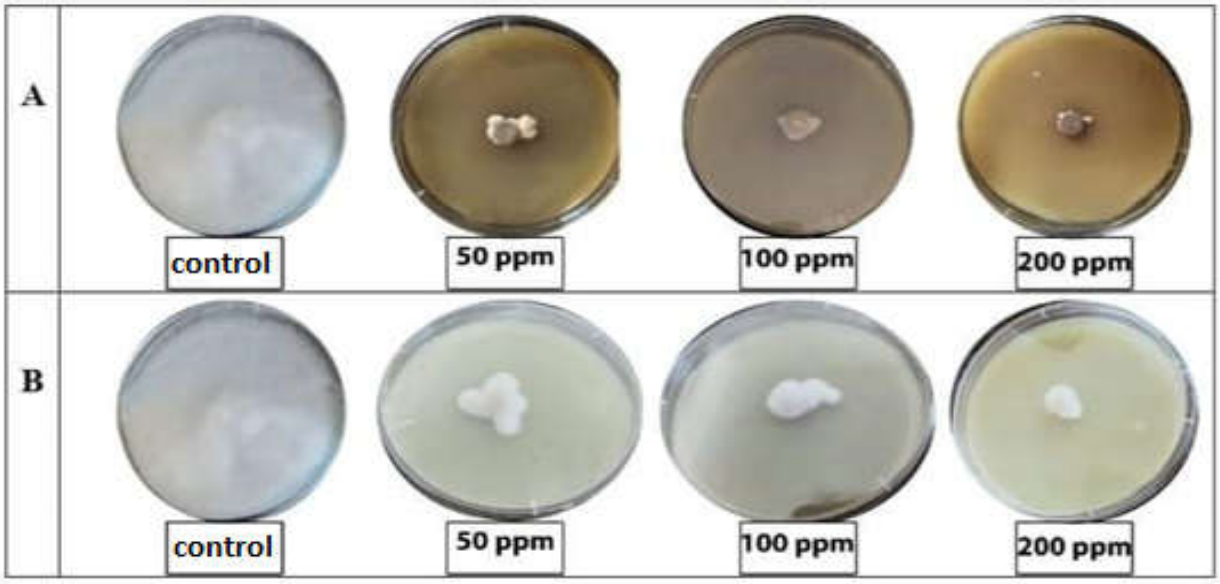


**Fig 4.** Inhibition of Fusarium growth as affected by the application of different concentrations of two fungicides (**A:** Rhizolex- T and **B:** Topsin-M).

### *Invivo* evaluation of tested (plant extracts , *Trichoderma* spp and fungicides) on the disease incidence:

During the pre-emergence stage, the infection with *F.oxysporum* f.sp. *phaseoli* inhibited the sowing of seeds by (66.70%) (Fig 1, D and Table, 6). Seed treatment with plant extracts, *Trichoderma* spp. and fungicides, suppressed the incidence of damping-off disease compared to the infected control under greenhouse conditions. As shown in Table 6, the highest reduction in pre-and post-emergence damping-off was attributed to Cumin. 10 % and Rhizolex-T100 ppm. This was expressed in a higher percentage of survival plants (77.80 %). The lowest reduction was attributed to *T.viride* (33.33 %). Also, the application of all treatments significantly

decreased the severity of bean wilt disease incidence. Rhizolex-T100 ppm and Cumin. 10 % gave the best results were at the highest concentration (2.9 and 3.7 % respectively). The non-treated control resulted in 92.6% infection severity.

**Effect of tested (plant extracts , *Trichoderma* spp. and fungicides) on plant growth parameter:**

Vegetative growth of bean plants cultivated in the infested soil with *Fusarium oxysporum* f.sp *phaseoli* was positively improved by the application of various plant extracts (Table 7). The obtained results show that Cumin and Carnation plant extracts were also the best for growth parameters, in comparison with control. Plant height, number of branches, and leaves per plant were recorded more than twice as much as control plants when these extracts were used at a 10% concentration. Though, Tumeric and Pomegranate extracts gave the lowest efficiency.

Results given in Table (7) indicate that *T. harzianum* and then *T. asperellum* were the best bioagents that improved bean plant height, average number of branches, and leaves per plant. In this request, *T. viride* showed the least efficiency. However, all tested *Trichoderma* isolates improved bean growth parameters significantly, in comparison with control treatment. Also, results present in Table (7) demonstrate that both commercial fungicides significantly increased the plant height, number of branches and number of leaves per plant at 100 ppm concentration compared to the infected control.

**Table 6.** Efficacy of bioagents and fungicides against Fusarium wilt incidence (*in vivo*).

| Treatments | | Disease incidence (%) | | | |
|---|---|---|---|---|---|
| | | Pre-emergence (%) | Post-emergence (%) | Survived plants (%) | Severity of infection (%) |
| Plant extracts | Chili Pepper .10% | $22.22^{d}$ | $11.11^{b}$ | $66.70^{b}$ | $7.4^{e}$ |
| | Tumeric. 10 % | $33.33^{c}$ | $22.22^{a}$ | $44.44^{d}$ | $14.2^{c}$ |
| | Carnation. 10% | $11.11^{e}$ | $22.22^{a}$ | $66.70^{b}$ | $4.1^{fg}$ |
| | Cumin. 10 % | $11.11^{e}$ | $11.11^{b}$ | $77.80^{a}$ | $3.7^{fg}$ |
| | Pomegranate.10% | $33.33^{c}$ | $11.11^{b}$ | $55.55^{c}$ | $11.1^{d}$ |
| *Trichoderma* spp. | *T.harizianum* | $22.22^{d}$ | $22.22^{a}$ | $55.55^{c}$ | $12.4^{cd}$ |
| | *T.viride* | $44.44^{b}$ | $22.22^{a}$ | $33.33^{e}$ | $18.5^{b}$ |
| | *T.asperellum* | $44.44^{b}$ | $11.11^{b}$ | $44.44^{d}$ | $14.2^{c}$ |
| Fungicides | Rhizolex-T100 ppm | $11.11^{e}$ | $11.11^{b}$ | $77.80^{a}$ | $2.9^{g}$ |
| | Topsin-M 100 ppm | $11.11^{e}$ | $22.22^{a}$ | $66.70^{b}$ | $5.3^{ef}$ |
| Control (Infected) | | $66.70^{a}$ | $11.11^{b}$ | $22.22^{f}$ | $92.6^{a}$ |
| Control (healthy) | | 0 | 0 | 100 | 00 |
| L.S.D 0.05 | | 1.72 | 1.76 | 1.03 | 2.17 |

Means within the same column followed by the same letter(s) are not significantly different (P≥ 0.05, LSD tes.

**Table 7.** Efficacy of different on bean growth parameters grown in infested soil with Fusarium wilt incidence under greenhouse conditions (*in vivo*)

| Treatments | | Vegetative parameters | | |
|---|---|---|---|---|
| | | Plant height (cm) | No. of branches / plant | No. of Leaves / plant |
| Plant extracts | Chili Pepper (10%) | $32.1^{de}$ | $6.2^{bc}$ | $31.6^{d}$ |
| | Turmeric (10 %) | $21.1^{i}$ | $4.3^{f}$ | $23.0^{hi}$ |
| | Carnation (10%) | $36.4^{bc}$ | $6.0^{bcd}$ | $31.0^{d}$ |
| | Cumin (10 %) | $38.0^{b}$ | $6.7^{ab}$ | $37.3^{b}$ |
| | Pomegranate (10%) | $26.1^{gh}$ | $5.3^{de}$ | $24.3^{gh}$ |
| *Trichoderma* spp. | *T.harizianum* | $33.6^{d}$ | $6.3^{bc}$ | $35.7^{c}$ |
| | *T.viride* | $29.8^{ef}$ | $5.8^{cd}$ | $28.7^{e}$ |
| | *T.asperellum* | $31.0^{e}$ | $6.3^{bc}$ | $35.6^{c}$ |
| Fungicides | Rhizolex-T 100 ppm | $40.2^{a}$ | $7.1^{a}$ | $42.5^{a}$ |
| | Topsin-M 100 ppm | $34.3^{cd}$ | $6.7^{ab}$ | $37.3^{d}$ |
| Control (Infected) | | $15.8^{k}$ | $3.4^{g}$ | $13.3^{j}$ |
| L.S.D. 0.05 | | 2.21 | 0.805 | 1.403 |

Means within the same column followed by the same letter(s) are not significantly different (P≥ 0.05, LSD test)

## Discussion

In recent years, scientists have been drawn to naturally occurring plant products as potential sources of novel fungicides that could serve as safe alternatives to synthetic compounds. Several formulated botanical extracts were shown to effectively reduce soil-borne *F. oxysporum* and increase symptomless plants in controlled experiments. In this investigation, we used some plant extracts to control *F. oxysporum* f.sp. *phaseoli* under laboratory and greenhouse conditions.

In the current study, *invitro* assays revealed that aqueous extracts of Cumin, Carnation, Chili Pepper, Pomegranate peel, and Tumeric had an effective inhibitory effect on *F. oxysporum* f.sp. *phaseoli* mycelium growth. *Cuminum cyminum* (Cumin) had the most potent fungal inhibitory effect against Fusarium growth when compared to the other plant extracts tested. Several studies have used plant extracts to combat soil-borne pathogens. For example, Obongoya *et al*. (2009) found that cumin extracts completely inhibited 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*.

*Punica granatum* has been documented to posse's fungitoxic characteristics and has been able to control (*In vitro*) a number of fungi. Studies on the fungitoxic features of *P. granatum* on *Sclerotium rolfsii, Fusarium oxysporum, Colletotrichum* sp. and *Aspergillus niger* showed that it inhibited spore germination and mycelial growth in some of the tested fungi (*In vitro*) (Dissanayake 2014).

On the other hand, antagonistic fungi, specifically *Trichoderma* spp., have been widely used against *Fusarium* spp. In the present study, three *Trichoderma* spp. isolates were obtained from the rhizosphere of healthy bean plants. These were identified as *T. harzianum, T. asperellum,* and *T. viride,* which were also tested against the pathogen under study.

Based on the paired culture tests, three *Trichoderma* spp. isolates may have several modes of action in the inhibition of colonial growth of *F. oxysporum* such as competition for nutrients and space, mycoparasitism, and antibiosis, as reported by Alabouvette *et al.,*(2009). *T. harizianum* and *T. asperellum* overgrew above the *F. oxyporum* f. sp.

*Phaseoli* colonies, degrading their mycelium due to its antagonistic properties against the pathogen. Similar results were obtained by Otadoh *et al.,* (2011). While *T. viride* an induce inhibition zone between the pathogen and *T. viride. Trichoderma* spp. has biocontrol potential because of several qualities which include antagonism, antibiotics and degrading enzymes that digest the cell wall, and similar results were stated by Sharma *et al*. (2009). Barakat *et al*. (2007) reported that this activity could also be recognized as the ability of *Trichoderma* spp. to develop direct exchanges with pathogens to produce antimicrobial substances since mycoparasitism complicated physical, attached by synthesis of hydrolytic enzymes and antibiotics.

In addition, the current study demonstrated that both tested fungicides (Rhizolex-T® and Topsin-M®) significantly reduced the mycelium growth of *Fusarium*. Increasing the concentration of both tested fungicides showed more efficiency in reducing fungal growth. Such findings are consistent with those of Jat *et al*., (2017), who reported that the fungicides Topsin-M®, Rhizolex-T®, and Tecto® 500 SC had a significant suppression efficiency when used against *F. oxysporum* f.sp. *corianderii invitro*.

Moreover, the application of plant extracts as seed coating revealed their efficacy against seed or plant invasion under greenhouse (*in vivo*) conditions, which resulted in a significant reduction in the wilt incidence of bean plants. The suppression of wilt development under greenhouses seems to be in correspondence with the ability of these plant extracts to reduce disease incidence. Cumin extract was the most effective tested extract in reducing wilt disease and improving plant growth, under greenhouse and artificial soil conditions. These findings are also in accordance with Raza *et al*., (2017). The application of three *Trichoderma* spp. isolates to the infested soil with *F. oxysporum* showed a significant reduction in disease incidence and plant growth. The most effective treatment was reported when *T. harzianum* and *T.asperellum* improved individually applied to Fusarium treatments. Similar results were also reported by Vandna and Priya (2014) and Al-Ameiri (2015). Besides the antifungal activity of biocontrol agents, Hafez *et al.,* (2012) reported that the bioagents activate pathogenesis-related protein synthesis before the pathogen invades the host plant, which has a direct impact on decreasing the ability of the pathogen to cause wilt and root-rot diseases. Zehra *et al*., (2017) indicated that the defense-related proteins and phenolic compounds were found to be increased several folds at different time intervals following the combined treatment of biological and chemical inducers. Many years of increasing use of chemicals have created a situation leading to an ecological imbalance and the increase of multiple multi-resistant pathogenic microorganisms (El-Mougy *et al*., 2007).

## Conclusions

Our study demonstrated that the application of aqueous plant extracts could be an applicable, safe, and cost-effective method for controlling soil-borne diseases (*F. oxysporum* f.sp. *phaseoli)* compared to synthetic fungicides. Also, the use of plant extracts in agriculture as fungicides has advantages as they disintegrate in nature and do not leave a toxic residue on plants. Also, our study reported that local isolates of *T.harzianum, T.asperellum* and *T.viride* have the potential for use as biological control agents to protect bean plants from *F. oxysporum* f.sp. *phaseoli.* Other practices could be integrated into biocontrol programming aiming to improve crop health and provide new standards of disease management where other techniques are inefficient.

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