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Effect of Some Biocontrol Factors and Their Efficacy in Resistance to Fusarium Wilt Disease Caused by *Fusarium oxysporum* F.sp. *Cucumerinum* on Cucumber Plant Under Open Field Conditions

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Abstract. The aim of this study is to evaluate the efficacy of some biological control agents in the resistance of *Trichoderma harzianium, Trichoderma viride* and mycorrhizal fungi *Glomus mosseae*, individually or collectively, *Fusarim oxysporum f.sp. cucumerinum*. The results of this study showed that the combined biological control factors had a positive and significant effect in reducing the severity of injury of the disease by 20% and 21% respectively, with an effect efficiency of 73.92% and 65.30% in reducing the percent of the injury severity. Followed by the treatment of mycorrhizal fungi *G. mosseae*, the results showed a reduction of injury severity with 26.7%, 23% and 65.18%, 62.47%. The use of *T.harzianium* and *T. viride* has also resulted in a significant reduction of the injury severity by 43.3%, 23%, 46.7%, 29.3%, 46.7% and 23%, respectively. In addition, the use of these biological control agents had a significant impact on the growth. An increase in the plant height, leaves number, wet and dry weight of plant was also observed.

INTRODUCTION

Fusarium wilt is a disease caused by *Fusarium.oxysporum* f. sp. *cucumerinum* and it is one of the most threatening diseases that cause significant economic losses in cucumber plants, especially in warm seasons when soil temperature and humidity are relatively high. Martínez *et. al* [1] and Yuan *et. al* [2] reported that the fungus *F.oxysporum* f. sp. *Cucumerinum* is the main cause of wilt disease in cucumber plants. Symptoms of wilt starts with the yellowing of leaves and growth of fungus mycelium colonies on the stem of the effected plant in the advanced stages of infection. Therefore, causing the collapse of internal tissues. The disease does not rot roots of the infected plants with Fusarium vascular disease in the early stages [3]. The results of the field samples showed the prevalence of fusarium wilt disease on the cucumber in all survey areas in Babil province which ranged between 37-11% [4]. According to the literature, the biological control against fungal diseases has made a significant progress in recent years for the efficient use of natural resources against harmful organisms, especially at the root zone and soil [5]. *T. harzianum* has shown high capability in reducing the incidence of wilt disease caused by fungus *F.oxysporum* f. sp. *cucumerinum.* Srinon *et. al* [6] reported that *Trichoderma* spp. fungi was able to inhibit growth of the pathogen fungi. The study [6] also

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highlighted the importance of fungal parasitism by production many enzymes assisting to dissolve harmful compound such as, cellulose and hemicellulose.

Similarly, Al-Tuwaigri [7] examined the impact of fungi in controlling the disease and increasing the height of the total vegetative and root as well as increasing the dry and wet weight of the plant. Al-Hujazy [4] reported that the use of *T. harzianum* and *T. viride* as biological control had significantly reduced the proportion and severity of injury and also had increased the height, dry and wet weight of cucumber plant compared to the treatment of fungus *F.oxysporum* f.sp. *cucumerinum*. The Arbuscular mycorrhizal fungi has an effective role in plants by coexisting with their roots resulting in significant increase in vegetative and root weight, increased uptake of organic matter and salts of host plants as well as increased tolerance to environmental and biological stresses. These fungi can also be an important factor in biocontrol [8,9]. Studies have shown that the mycorrhizal fungi *Glomus mosseae* has an important role in enhancing the tolerance of plants to some pathogens, including the cause of wilt and root-rot diseases such as *Fusarium oxysporum, Rhizoctonia solani* Kühn, *Phytophthora* spp. and *Pythium* spp. [10,11]. The vaccination of cucumber seedlings with internal mycorrhizal fungi (VAM) had reduced the injury percent with *pythium ultimum* seedling disease [12] and stimulated the genes responsible for plant resistance to the production of Jasmonic acid (JA), ethylene (ET) or the production of salicylic acid (SA), and also plays a role in stimulating the production of conjugated proteins (PRPs) [13]. As a result of the great damage caused by the fungus in the growth and productivity of cucumber as well as the threat that the disease poses, this study is aimed to use biological factors *T. harziunium, T.virirde* and Mycorrhiza as one of the important strategies in reducing the damage of the disease while assessing the efficiency in disease control and its effect on some growth parameters.

MATERIALS AND METHODS

This study was conducted at College of Agriculture - University of Anbar-Abu Ghraib under open field conditions, the seeds of local cucumber were planted in cork dishes with each dish contains 120 holes filled with Peatmoss which was used as a seed germination medium. The field was divided into furrows of 5 meters in length. The 3 weeks old seedlings were the transferred and planted on the upper sides of the furrows after adding the treatments according to the dates of addition of each factor.

Preparation of the isolates of fungi used in the study

The fungal infection was prepared by isolating the fungus on the local millet seeds by placing 50 gm of seeds in a glass flask (250 mL) after soaking in water for 6 hours then sterilized using autoclave and was left to cool. Five tablets (0.5 cm diameter) of the PDA media, which was used to grow fungi *F. oxysporium,* were placed in each flask. After seven days, the flasks were incubated at 25 ±1 °C for 10 days with shaking for every two days to ensure the fungus was distributed evenly throughout the media. The artificial infection was conducted with a suspension of conidia spores which was taken from colonies of fungi (14-day old) at a concentration of 5×10^6 spore. ml^{-1}, After inflicting some wounds on the root surfaces and removing its bottom using a shaving blade, the samples were then soaked in the suspension of spores for 30 minutes before planting directly.

Preparation of *T. Harzianum* and *T. viride* suspension

A pure isolates of biocontrol was used on the PDA media and incubated at 25 ± 1 ° C for seven days. The fungal growth on petri dish (9 cm diameter) was added to 100 mL sterile distilled water with manual shaking and the concentration of the suspension was calculated using the counting slice. The biocontrol fungus was added with a concentration of $7x\ 10^7$ (colony formation units). mL^{-1}. Where 10 ml of suspension was added for each hole, and the treatment was conducted 24 hours before planting.

Preparation of mycorrhizal inoculum

The faba bean roots commensalism with Mycorrhiza fungus *G. mussaea* and the soil surrounding the root containing the Mycorrhiza spores, 10 gm of infection was used for each hole.

Studied characters

1. **Characters of vegetative growth:** These characters were studied since planting until the end of the study which included: Plant height [14], number of leaves per plant after 60 days of planting, wet and dry weight of vegetative and root mass after 70 days of planting.

2. **Calculation of severity of the infection:** The severity of the infection was calculated from the onset of the symptoms of the disease on the studied plants weekly after 21 days of planting until the end of the study (70 days). The hexagonal scale was used to determine the severity of the injury as follows: 0 = uninfected plant, 1 = 25% of plant leaves seems to wilt and yellowing , 2 = 26 - 50% of plant leaves seems to wilt and yellowing, 3 = 51 - 75% of plant leaves seems to wilt and yellowing, 4 = 76 - 100% of plant leaves seems to wilt and yellowing, 5 = dead plants. The severity of the infection was calculated according to the equation:

   R = severity of the injury % $\Sigma(a\ x\ b)$ = number of infection plants (a) multiplied by the reading scale. N,b= total number of plants, K= the highest degree of scale which equal 5. Testing the soil treatment with biocontrol agents and their role in controlling fusarium wilt disease and its impact on some growth parameters. After the preparation of the above research factors, the biological control agents were added to the soil three days before the transfer of the seedlings. The treatments included the following:

   a) Soil treated with *T. harzianum* + *F. oxysporium*
   b) Soil treated with *T. viride* + *F. oxysporium*
   c) Soil treated with Mycorrhiza + *F. oxysporium*
   d) Soil treated with *T. harzianum* + soil treated with *T. viride* + soil treated with Mycorrhiza + *F. oxysporium*
   e) Soil treated with *F. oxysporium*
   f) Soil not treated with fungus

RESULTS AND DISCUSSIONS

Biotic factors *T. harzianum, T.viride*, and Mycorrhizal fungus *G. mosseae* had demonstrated high ability and efficiency in reducing the percentage and severity of fusarium wilt disease caused by *Fusarium.oxysporum* f.sp. *cucumerinum* on cucumber (Table 1). While the percentage and severity of infection were 43.3, 23%, 46.7, 29.3%, 26.7, 23%, respectively, with the efficiency of 43.54,62.47%, 39.11, 52.20%, 65.18, 62.47%, the control treatment (inoculated with fungus only) had percentage and severity of injury of 76.7 and 61.3%. The results also showed that the use of biological control agents separately or combined had led to an increase of its effect on pathogenic fungi, where the percentage and severity of pathogenic fungi infection reached 20, 21%, respectively, with its efficiency of use of 65.30%, 73.92. These results were in agreement with those of the reported findings by Ahn *et. al* [15] and Mahdy *et. al* [16] which refered to the effect of these factors on resistance to pathogens, when they were used separately or combined. The inhibitory effect of *T. viride and T. harzianum* may be attributed to fungal parasitism and competition for nutrients and the place, their production of antibiotics, and a number of antiviral enzymes for a number of fungi pathogenic, including *Fusarium* spp, as well as secondary metabolic products of biological factors and their effective role in the induction of the plant defense system [16,17]. Mycorrhizal fungi works to stimulate the secretion of growth materials and regulators that stimulate their resistance. The mycorrhiza also makes some nutrients, such as phosphorus, more available to the plant, resulting in a good vegetative growth of the plant that makes it more tolerable to the pathogen [9,13].

The biological control factors were more efficient in increasing the plant height, leaves number, dry and soft weight of plant when used together which reached 74.3 cm, 22.33 leaves per plant, 21.03 gm, 198.7 gm, respectively (Table 2). The same biological control factors also showed an increase in plant height, number of leaves per plant, dry and wet weight of plant when used separately. The most important factors were the mycorrhizal fungi *Glomus* spp. The studied characters reached 69,60 cm, 20 leaves per plant, 20,27 gm, 187.7 gm respectively. The results indicated that all biological control factors alone or in combination were significantly reduced compared to control treatment that inoculated with fungus only with 39,90 cm, 16 leaves per plant, 18.3 gm and 127,40 gm, respectively. The increase in growth parameters was due to the ability of these factors to stimulate the induced systemic resistance by stimulating

the genes responsible for the resistance and improving its growth through the creation of growth regulator and proteins, increasing the availability of nutrients and increasing the plant tolerance to external stresses [8,13].

TABLE 1. Effect of biological control factors and its efficiency in controlling the fusarium wilt disease caused by *Fusarium.oxysporum* f. sp. *cucumerinum*.

Treatment	Infection percent %	Infection severity %	Used factor efficiency in reduction infection percent%	Used factor efficiency in reduction severity percent%
T. harzianium +F.oxysporium	43.3	23	43.54	62.47
T. viride +F.oxysporium	46.7	29.3	39.11	52.20
G.mossa +F.oxysporium	26.7	23	65.18	62.47
T.harzianum+T.viride+G .mossa+F.oxysporium	20	21	73.92	65.30
F.oxysporium	76.7	61.3		
Control (inoculated)	0	0		
L.S.D (0.05)	16.16	3.36		

*Each number represents an average of three replicates.
*In each replicate five plants.

TABLE 2. Effect of biological control factors in some growth parameters.

Treatment	Plant length (cm)	Leaves number per plant	Wet weight (g)	Dry weight (g)
T. harzianium +F.oxysporium	76.5	18	176.1	14.53
T. viride +F.oxysporium	65.5	18.33	169.5	16.00
G.mossa +F.oxysporium	69.6	20	187.7	20.27
Th+Tv+Gm+Fo	74.23	22.33	198.7	21.03
F.oxysporium	39.90	13	67.4	8.30
Control (inoculated)	76.10	21.67	209.0	23.90
L.S.D (0.05)	1.96	2.24	38.86	1.50

*Each number represents an average of three replicates.
*In each replicate five plants.

CONCLUSION

Biocontrol factors (*T. harzionum* , *T. viride , G. mosseae)* were generally used to reduce the disease infection and disease severity of fusarium wilt in cucumber plant. An increase of plant length ,dry weight , fresh weight and flower number were also observed on the treated samples.

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