Effect of application time on the efficacy of *Trichoderma* spp. to biologically control sunflower charcoal rot

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Abstract: Sunflower (*Helianthus annuus* L.) is considered as one of the most important oilseed crops in Egypt and worldwide. It is being infected with many pathogens, among these pathogens *Macrophomina phaseolina* (Tassi) Goid the causal pathogen of charcoal rot is the most prevalent one, and responsible for severe economic losses on sunflower. Fourteen isolates of *M. phaseolina* were collected from naturally infected sunflower plants. Pathogenicity testes revealed that tested isolates varied significantly in their pathogenic capabilities. But all of the tested isolates were pathogenic and incited the symptoms of pre- and post-emergence damping-off as well as symptoms of charcoal rot. In this study, antagonistic capabilities of 26 isolates of *Trichoderma* spp. were investigated under both laboratory and greenhouse conditions. *in vitro*, *T. harzianum* (T8) and *T. hamatum* (T12) proved to have high antagonistic capability against *M. phaseolina* fungus with inhibition percentage of 62.13% and 61.33%, respectively. Furthermore, these two isolates proved to have a high ability to control charcoal-rot disease. Data of greenhouse experiments showed that application of *T. harzianum* (T8) and *T. hamatum* (T12) decreased charcoal rot disease severity by 30.33 and 24.16% respectively. Time of application played a critical role to increase the efficiency of *Trichoderma* spp. to control charcoal rot. In this experiment *Trichoderma* was implemented into soil at different application dates to study the effect of application date on the efficiency of bioagents to control charcoal rot. Results of this experiment showed that the highest reduction in disease severity occurred when *T. harzianum* (T8) was applied seven days before soil infestations with *M. phaseolina* (38.40%). Data also demonstrated that application of either *T. harzianum* or *T. hamatum* led to significant increases in the percentage of survival plants with 72.5% and 68.33%, respectively. This study suggests using *Trichoderma* spp. could be an efficient method to control sunflower charcoal rot.

Keywords: Sunflower, *Macrophomina phaseolina,* charcoal rot, *T. harzianum, T. hamatum.*

تأثير ميعاد المعاملة على فعالية *Trichoderma* spp. لمقاومة العفن الفحمي في دوار الشمس بيولوجيا

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الملخص: دوار الشمس يعتبر واحداً من أهم المحاصيل الزيتية في مصر والعالم . يصاب دوار الشمس بالعديد من الممرضات. ويعتبر فطر *Macrophomina phaseolina* مسبب مرض العفن المن أكثر تلك الممرضات شيوعاً ، ومسئولاً عن خسائر اقتصادية كبيرة. في هذه الدراسة تم الحصول على 14 عزلة من الفطر الممرض من نبات مصاب طبيعياً بهذا الفطر. اختبارات القدرة المرضية أظهرت، بان تلك العزلات اختلفت من حيث قدرتها المرضية، وتسببت فى ظهور أعراض موت البادرات قبل وبعد الظهور فوق سطح التربة بالإضافة إلى أعراض العفن الفحمي.

تم تقييم القدرة علي التضاد لستة وعشرون عزلة مختلفة للفطر *Trichoderma* spp. ضد الفطر الممرض *M. phaseolina* معملياً. وأوضحت النتائج أن العزلتين؛ *T. harzianum* (T8) و *T. hamatum* (T12) لهما تأثير مضاد عالي ضد الفطر *M. phaseolina* في المعمل بنسب تثبيط بلغت 62.13% و61.33% على التوالي . وقد أظهرت هاتان العزلتان أن لهما قدرة عالية على مكافحة مرض العفن الفحمي. أظهرت نتائج التقييم تحت ظروف الصوبة أن استخدام *T. harzianum* و *T. hamatum* أدى الي تقليل شدة الإصابة بمرض العفن الفحمي بنسب بلغت 30.33 و 24.16% على التوالي, تم الحصول على أعلى خفض في شدة مرض العفن الفحمي (بنسبة 38.40%) عند معاملة التربة بالفطر *T. harzianum* قبل سبعة أيام من عدوي التربة بالفطر الممرض. علاوة على ذلك؛ اظهرت النتائج أن إضافة اى من الفطرين *T. harzianum* أو *T. hamatum* أدي إلى زيادة نسبة النباتات السليمة بنسب بلغت 72.5% و 68.33% على التوالي. هذه الدراسة تقترح أن استخدام *Trichoderma* spp يمكن أن يكون وسيلة فعالة لمقاومة مرض العفن الفحمي في دوار الشمس.

الكلمات المفتاحية: دوار الشمس ، العفن الفحمي .

1. Introduction

Sunflower *(Helianthus annuus* L.) is a member of *Asteraceae* family, and it is considered an important global oil seed crop. Sunflower is characterized by it's short-season crop (90–100 days), and this allows farmers sometimes to grow sunflower twice a year, [1]. Unfortunately, sunflowers are being infected by many bacterial, fungal, viral pathogens. Among these pathogens *Macrophomina phaseolina* (*M. phaseolina*) is a devastating pathogen in Egypt and worldwide. *M. phaseolina* can infect wide host range, as it causes charcoal rot disease in approximately 500 plant species in more than 100 families [2 , 3], The pathogen is infecting plants systematically especially in roots and basal internodes.

Symptoms of charcoal-rot typically appear at the end of the growing season, and include stunting, chlorosis and wilting are common symptoms. [4], which lead to severe reduction in sunflower production [5]. This reduction in yield is due to the ability of pathogen to impede the transport of water and nutrients to the upper parts of the plant, thus weakening the growth of the plant. *M. phaseolina* may kill plants prematurely as well as reduce yield especially in crops growing in high temperature and drought stress conditions [6]. There are several techniques currently used to manage *M. phaseolina* fungal infections. Chemical control has been intensively applied to control these destructive diseases, but the excess use of fungicide usually leads to undesirable effects, including their adverse effects on human and animal health, and emergence of new pathogenic strains resistant to the traditional fungicide. Thus, there is an urgent need to establish a new alternative method to control this destructive disease. Biological control can constitute this alternative method to control charcoal rot disease in sunflower. Biological control could be an efficient component of integrated management strategy of plant diseases, and can be used against a wide range of plant pathogens, but the type of bioagent and application methods varied according to pathogen. Biological control of plant pathogens is based on using specific microorganism to decrease inoculums density of the pathogen or reduce its virulence [7 , 8].

Implementation of bio-agents has proved to be an efficient method to manage many fungal, bacterial and viral pathogens during the last three decades [9]. Several species of *Trichoderma* spp. have been widely used in agricultural applications due to their effectiveness to reduce the disease severity of many pathogens especially against soil-borne fungi [10, 11, 12]. This high effectiveness of *Trichoderma* spp. to control plant pathogens is due to their reproductive capacity, their ability to survive in different climate conditions, depending on different kind of nutrients, their ability to colonize plant roots robustly [13]. These Features made *Trichoderma* spp. a ubiquitous bioagent present in almost all habitats. Several mechanisms have been proposed to explain the efficiency of *Trichoderma* spp. to control plant diseases and enhance the plants, for

example [14] revealed that *Trichoderma* spp. produce koninginins which has inhibitory effects on the growth of certain pathogenic microorganisms, and thus can reduce the occurrence of plant diseases. Koninginins molecules produced by *Trichoderma* spp. by which inhibited the growth of *Fusarium* spp. [15]. Another mechanism *Trichoderma* can reduce the disease severity of the pathogen is through producing secondary metabolite named harzianolide [16]. As harzianolide had an influence on the early stages of plant growth and promote root length and root tips, leading to better root development.

Several attempts have been made to biologically control charcoal rot under greenhouse and field conditions including treating sunflower seeds with *Trichoderma harzianum* [17], and to reduce charcoal rot in melon under greenhouse [18]. While soil application of *Trichoderma viride (T. viride)*, *Trichoderma harzianum* (*T. harzianum*) and *T. polysporum* significantly reduced the root-rot of Egg-plant under field conditions [19], and *T. harzianum* proved to be one the most efficient microorganisms for the biocontrol of *Rhizoctonia solani* infecting many hosts [20].

This study is attempting to utilize several species of *Trichoderma* spp. in order to biologically control charcoal rot disease caused by *M. phaseolina* under both laboratory and greenhouse conditions.

2. Materials and methods

2.1 Isolation and identification of the causal pathogen

Sunflower plants showing typical symptoms of charcoal rot were collected from different localities of Assiut Governorate, namely: Abuteeg, Assiut, Manfalout and Alkosia during June and August 2021. Diseased plants were uprooted, washed thoroughly under running water to remove any adherent soil particles, the diseased plant then were cut into small pieces about 1 cm each, and soaked in 2% sodium hypochlorite solution for 2 min. The remaining pieces were placed in Potato Dextrose Agar medium (PDA) and incubated at 28±2 °C for 2-4 days. Developing fungi were purified using hyphal-tip isolation technique described by [21] and were kept at 5 °C in the refrigerator for further

investigations. Fungal isolates were identified according to their morphological and microscopic characteristic as described before in [22, 23, 24]

2.2 Pathogenicity tests

The ability of fourteen isolates of *Macrophomina phaseolina* to cause the symptoms of charcoal rot were tested on Giza-102 sunflower cultivar at the greenhouse of Plant Pathology Department, Faculty of Agriculture, Assiut University.

Inocula of *Macrophomina phaseolina* isolates were prepared by growing the pathogens on sterilized barely grain medium (150 g barley + 50 g clean sand + 4 g sucrose + 0.2 g yeast + 200 ml water) and inocubated at 27 °C for 15 days. Sterilized pots (30 cm in diameter) containing sterilized clay-sand soil (2: 1) were infested with equal amounts of *M. phaseolina* inoculum of one of the fourteen tested isolates at the ratio of 1% of soil weight, mixed well and thoroughly irrigated. This step was repeated with all fourteen tested *M. phaseolina* isolates. Pots containing barley medium only without any pathogen were used as control. Sunflower seed (Giza -102 cultivar) were surfacely sterilized, and 10 sunflower seeds were planted in each pot. Four pots were used as replicates. Experiments were carried out under greenhouse conditions; the plants were irrigated when it was necessary and plants were observed on a daily basis to monitor and record the development of the infection.

2.3 Disease assessment

In order to determine the pathogenic capabilities of each one of the fourteen tested *M. phaseolina* isolate, Sunflower pre- and post- emergence damping-off were recorded in pots challenged with pathogenic fungi 15 and 30 days after post-sowing, respectively. 75 days after planting date, the disease severity of charcoal-rot was determined visually using disease index from 1-10 to determine the severity of charcoal-rot disease symptoms according to [25] as follows:

(0)= No symptoms on plant;
(1)= 1-<10 % area infected;
(2)= 10-<25 % area infected;
(3)= 25-<50 % area infected;
(4)= 50-<75 % area infected;
(5)= 75-≤100 % area infected or dead plant.

The Disease severity was calculated using the following formula:

$$\frac{\sum[(N\times 0)+(N\times 1)+.....(N\times 5)]}{TN}$$

(N) refers to number of plants in each grade of the disease scale; and (0, 1, 2, ... 5) are numerical grades of disease severity scale. TN: refers to total number of plant multiplied by the highest grade in disease scale

2.4 Isolation and identification of *Trichoderma* spp.

To biologically control *M. phaseolina* the causal pathogen of Charcoal rot. Trichoderma were isolated from the soil and rhizosphere of healthy sunflower plants. Isolation was carried out according to the method of [21] as described above and kept at 5 °C for further investigations. Trichoderma isolates were identified according to their morphological characteristics as described by [24].

2.5 Preliminary tests for antagonistic capability of *Trichoderma* to inhibit *M. phaseolina in vitro*

Antagonistic capability of twenty-six isolates belonging to *T. harzianum, T. hamatum, T. viride* and *T. koningii* were tested against the highly pathogenic isolate of *Macrophomina phaseolina* (M10), which showed the highest ability to cause severe symptoms of charcoal rot *in vitro.* Petri dishes (9 cm in diameter) each containing PDA media were inoculated in one side with *M. phaseolina*, while one of the 26 *Trichoderma* isolates were placed in the opposite side. Petri dishes inoculated with *M. phaseolina* alone served as control. Five replicates were used in case of each *Trichoderma* isolate and all plates were incubated at 28±2 °C till the mycelia in the control covered the plates completely, and then the percentage of mycelial inhibition was calculated according to the following formula:

Growth Inhibition (%) = $\frac{A-B}{A}\times 100$

wherea (A) refers to mycelial growth in control plates; (B) refers to mycelial growth in pathogen challenged with one of the *Trichoderma* isolates.

2.6 Application of *Trichoderma* isolates to biologically control *sunflower charcoal rot* under greenhouse conditions

In order to assess the efficiency of certain *Trichoderma* isolates to control sunflower charcoal rot under greenhouse conditions, the most efficient *Trichoderma* spp. (*T. harzianum* (T8) and *T. hamatum* (T12),) which revealed the highest inhibition percentage *in vitro* against *M. phaseolina* were selected to be applied *in vivo*. Inocula of the highly pathogenic isolate of *M. phaseolina* (M10) were prepared as mentioned above and soil was infested at the ratio of 1% of soil weight before planting date. While, *Trichoderma* bioagents was added to soil in form of spore suspension which was prepared through inoculation of each bioagents in 250 ml conical flasks containing 50 ml of Potato Dextrose Broth and incubated for two weeks at 28±2 °C in the dark. Then the culture were centrifuged and the supernatant was discarded, and the spore pellet was re-suspended in sterilized water, and the spore suspension was adjusted at ($5x10^5$ conidia per ml), and then 50 ml suspension of *Trichoderma* isolates were applied to each pot which previously was infested with the highly pathogenic *M. phaseolina* (M10). 10 seeds of *Giza-102 sunflower* cultivar were sown in each pot and arranged in complete randomized design. Positive control was inoculated with *M. phaseolina* only without any bioagents, while pots containing 1% barley medium only were used as negative control. There were four pots per treatment.

2.7 Effect of application time on the efficiency of *Trichoderma* sp. to control charchoal rot

To evaluate the effect of application time of *T. harzianum* and *T. hamatum,* to control *M. phaseolina* the causal pathogen of charcoal rot in sunflower, the bioagents were applied at three different times as described by [26]; first: 7 days before soil infestation with *M. phaseolina*, second: soil was infested with

bioagent and pathogenic fungi at the same time, third: soil was first infested with *M. phaseolina* and after 7 days, the bioagent was added to the soil. Experiment was repeated twice during the summer of 2022 and 2023. Investigation was conducted under greenhouse conditions at Department of Plant Pathology, Faculty of Agriculture, Assiut University. The disease severity of charcoal rot was estimated after 10 weeks post-sowing, as mentioned above in pathogenicity tests.

3. Statistical analysis

Obtained results were analyzed using ANOVA test and the mean differences were regarded as significant using L.S.D. test at 5% level of probability according to [27].

4. Results

4.1 Identification of the causal pathogen

Collected isolates were identified as *Macrophomina phaseolina* based on the morphological and microscopic characteristics of mycelium and spores according to [22, 23, 24 .

4.2 Pathogenicity tests

The pathogenic capabilities of 14 isolates of *M. phaseolina* were evaluated under greenhouse conditions. Data presented in Table (1) showed that all tested isolates were pathogenic and led to occurrence of pre- and post- emergence damping-off as well as inducing varied degrees of charcoal rot symptoms. However, these tested isolates differed significantly in their pathogenic capacity. Such results confirm that *M. phaseolina* is the causal pathogen of sunflower charcoal-rot disease in Upper Egypt.

The highest percentage of pre-emergence damping off in seedlings of Giza-102 sunflower cultivar occurred by isolate No. M10 (42.50%), followed by isolate No. M8 (40%) while isolates No. M1 and M13 produced the highest percentage of post-emergence damping off (35% and 27.50%), respectively.

Obtained results indicated that *M. phaseolina* isolates (M10, M13, M9 and M11) were the most harmful among all tested isolates, and caused plant mortality at seedling stage with rate higher than or equal to 50%. The most severe isolate was M10 followed by M13, M9 and the latter has the same severity of M11.

Other tested isolates differed in their virulence, but they also caused significant disease severity in sunflower plants compared with control (healthy) plant , but their pathogenic capacity were less than the aforementioned isolates. However, the least capable *M. phaseolina* isolates to incite charcoal rot symptoms on sunflower were isolates M7, M5 and M6 respectively.

4.3 Identification of Trichoderma isolates

Twenty-six isolates of *Trichoderma* spp. were isolated from the soil and rhizosphere of healthy sunflower plants (Table- 2). Isolates were identified according to their morphological and characteristics of mycelia and conidiophores as described by [24]. The obtained isolates were: nine isolates of *Trichoderma harzianum* Rifai, seven isolates of *Trichoderma hamatum* (Bonord.) Bainier, four isolates of *Trichoderma viride* Pers, and six isolates of *Trichoderma koningii* Oudem.

4.4 Preliminary test for efficacy of *Trichoderma* spp. against *M. phaseolina* in- *vitro*

The antagonistic ability of twenty six isolates of *Trichoderma* spp. (9 isolates of *T. harzianum,* 7 isolates of *T. hamatum,* 4 isolates of *T. viride* and 6 isolates of *T. koningii*) against *M. phaseolina* were determined *in vitro*. Data presented in Table (2) proved that all four Trichoderma species significantly inhibited the growth of *M. phaseolina*, but the percentage average of growth inhibition differed from one species to another. The highest inhibition in *M. phaseolina* growth occurred in case of *T. harzianum* (55.50%) followed by *T. hamatum* (53.87%), *T. viride* (53.20%) and *T. koningii* (44.45%). *T. harzianum* isolate No. T8 followed by *T. hamatum* isolate No. T12 caused the highest reduction in *M. phaseolina* growth with inhibition of 62.13 and 61.33% respectively (Figure 1) while the least reduction was obtained by *T. koningii* (T25) with inhibition of 40.62%.

4.5 Bio-control efficacy of *Trichoderma* isolates against *sunflower charcoal rot* under greenhouse conditions

Isolates of *T. harzianum* (T8) and *T. hamatum* (T12) which revealed high potential to inhibit the growth of *M. phaseolina in vitro* were selected to evaluate their capacity to reduce the incidence of sunflower damping-off and charcoal-rot under greenhouse conditions. Data presented in Table (3) indicated that *M. phaseolina* (M10) was able to infect sunflower plants and reduce the percentage of surviving plants. It was able to cause significant increase in pre- and post-

emergence damping-off after 2 and 4 weeks compared with uninfected control. Whereas, application of *Trichoderma* isolates gave the reverse effect, they significantly increased the survival rate of sunflower plants and taken-down the percent of mortality in plants compared with the infected control. Application of *T. harzianum* or *T. hamatum* increased the percentage of survival plants with 72.5% and 68.33%, respectively. The bio-agents were applied at three different times; first: 7 days before challenging with *M. phaseolina*, second: at the same time with challenging with *M. phaseolina*, and third: 7 days after challenging with *M. phaseolina*.

Data also revealed that, both *T. harzianum* (T8) and *T. hamatum* (T12) significantly reduced the disease severity of *M. phaseolina*. Higher reduction in average disease severity of charcoal rot (30.33%) was observed in case of application of *T. harzianum* (T8), while reduction in disease severity of charcoal rot was 24.16% when *T. hamatum* (T12) was applied (average of three different application times). The data clearly showed that the application time of bio-agent significantly affects the efficiency of both *T. harzianum* and *T. hamatum* to control charcoal rot disease. The data indicated that the highest reduction in charcoal rot severity was obtained when *T. harzianum* or *T. hamatum* were applied 7 days before challenging with *M. phaseolina* (38.40% and 27.18%, respectively). In contrast, the efficiency of either T8 or T12 was significantly reduced when they were applied either at the same time with *M. phaseolina* or 7 days after challenging with *M. phaseolina* (23.81% and 20.07%, respectively). In general, success of the bio-control agents in controlling the soil borne pathogenic fungi depends on many factors i.e., the ability of the antagonists fungi to penetrate the root tissues faster than the pathogen and the activity of the microorganisms to absorb the nutrients from the soil faster than the pathogen [28, 29] . In consequence, in this study we found that the best method to control sunflower charcoal rot is application of *T. harzianum* (T8) followed by *T. hamatum* (T12) 7 days before challenging with *M. phaseolina.*

5. Discussion

Sunflower is an important oil crop in Egypt and worldwide, and there is an urgent need to increase the productivity of sunflower in Egypt in order to fill the gap in oil consumption in Egypt. On way to increase sunflower productivity is through establishing efficient method to control sunflower diseases which cause severe losses in sunflower annual yield production. One of these diseases is charcoal-rot caused by *M. phaseolina*, which is considered among the most destructive pathogens infecting sunflower in Egypt, and worldwide. *M. phaseolina* led to severe losses in sunflower yield. Many traditional strategies are applied to control charcoal rot disease in sunflower, including chemical control, but this method raises a lot of alarm because their dangerous effects on environment [9]. Moreover, constant and excessive application of fungicides led to emergence of resistant fungal pathogens against the most common fungicides [30]. Plus application of fungicides is causing an undesirable effect on non-targets and beneficial organisms [31] Another, traditional method to control fungal disease is through growing a resistant cultivars against pathogen, but availability of these resistant cultivars, sometimes restrict their effectiveness to control plant diseases.

In these circumstances, using biological method to control plant diseases seems to be the most attractive solution to control charcoal rot diseases in sunflower. Biological control in general means application of certain beneficial organisms in order to control or restrict the life cycle of one or more plant pathogens. The most common bioagent, which is typicaly used to control plant pathogens is *Trichoderma* spp. which proved high ability to control many diseases.

Trichoderma sp. is famous for their ability to reduce disease severity of many diseases, as 90% of all attempts to control plant diseases by fungi are carried out by different strains of *Trichoderm* [32] and can be present in any habitat under different environmental conditions [33].

In this study, the potential use of *Trichoderma* isolates for management of sunflower charcoal rot disease was investigated. *Trichoderma* spp. were isolated from the soil and rhizosphere of healthy sunflower plants and proved their antagonistic effects against *M. phaseolina in-vitro* and *in-vivo*.

In-vitro, our results demonstrated that all tested *Trichoderma* isolates significantly inhibit the growth of *M. phaseolina in vitro. T. harzianum* (T8) and *T. hamatum* (T12) were the most capable isolates to inhibit the growth of *M. phaseolina in vitro* with average inhibition of 62.13% and 61.33%, respectively. Both *T. harzianum* (T8) and *T. hamatum* (T12) were applied under greenhouse conditions and proved to be highly efficient to reduce the disease severity of charcoal rot during two successive trials during the summer of 2022 and 2023. Results revealed that *T. harzianum* produced the highest reduction in diseases severity of charcoal rot with 30.33% while *T. hamatum* reduced the disease severity of charcoal rot with 24.16% (average of three different application times). These findings are in the line with previous findings reported by [7, 11, 34, 35] who mentioned that *Trichoderma* strains are very efficient to control several plant pathogens

Furthermore, we found that time application of the bioagents plays a critical role in determining the efficiency of either *T. harzianum* and *T. hamatum* to control charcoal rot of sunflower, the data of this study demonstrated that, the longer time the bio-agent was applied before infection, the better the efficiency of the bio-agent.

These results indicate that application of bio-agent require enough time to establish itself in the soil before competing with the causal pathogen. This result was confirmed before through findings of [26].

Several mechanisms have been suggested to explain the antagonistic capabilities of *Trichoderma* spp., this may include production of some antibiotics, which may play remarkable role in the biological control and mycoparasitism processes against plant pathogen. Or this antagonistic effect is

due to direct interaction between *Trichoderma* and *M. phaseolina* which involve synthesis of several hydrolytic enzymes in addition to some toxin which acts synergistically with the enzymes to inhibit the growth of *M. phaseolina* [35] Or due to secretion of some compounds which can deter the growth of *M. phaseolina* [36]. Some researchers have attributed the ability of *Tricoderma spp*. to control *M. phaseolina* to their ability to compete for nutrient [33] , or some *Trichoderma* spp. can produce highly efficient siderophores that made iron unavailable in soil and thus inhibit the growth of casual pathogen [37] as well as *Trichoderma* can enhance the growth of plants and can act as a biofertilizer through production of compounds that stimulate the growth and promote plant defense mechanisms [38] *Trichoderma* spp. can produce some toxins against plant pathogens like koninginins which has inhibitory effects on the growth of certain pathogenic microorganisms , and thus can reduce the occurrence of plant diseases [15]. Or producing secondary metabolite named harzianharzianolide [16], harzianolide had an influence on the early stages of plant growth and can promote root length and root tips, and led to better root development. In addition *Trichoderma spp.* can enhance the plant growth [14]..

6. Conclusion

Application of *T. harzianum* as well as *T. hamatum* proved to be a valuable method to control *M. phaseolina* the causal pathogen of charcoal rot of sunflower under greenhouse conditions. The highest reduction in disease severity was obtained when *T. harzianum* was applied seven days before soil infestations with *M. phaseolina* (38.40%). Further studies are required to study the potential effects of using *T. harzianum* as well as *T. hamatum to control M. phaseolina under field conditions.*

Acknowledgment

We would like to thank Department of Pant Pathology, Faculty of Agriculture , Assiut University for helping to conduct this study

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Table 1. Pathogenicity tests of *M. phaseolina* isolates on *Giza-102 sunflower* cultivar under greenhouse conditions.

***Macrophomina phaseolina* isolates No.**	**Geographic origins**	**Damping-off (%)**		**Survival plants %**	**Charcoal rot severity**
		Pre-emergence	**Post-emergence**		
M1	**Abuteeg**	**07.50**	**35.00**	**57.50**	**3.72**
M2		**25.00**	**15.00**	**60.00**	**3.60**
M3		**15.00**	**22.50**	**62.50**	**3.55**
M4		**37.50**	**12.50**	**50.00**	**3.67**
M5	**Alkosia**	**17.50**	**10.00**	**72.50**	**3.32**
M6		**20.00**	**10.00**	**70.00**	**3.45**
M7		**17.50**	**07.50**	**75.00**	**3.30**
M8	**Assiut**	**40.00**	**07.50**	**52.50**	**3.78**
M9		**37.50**	**12.5.0**	**50.00**	**3.83**
M10	**Manfalout**	**42.50**	**10.00**	**47.50**	**3.92**
M11		**32.50**	**17.50**	**50.00**	**3.83**
M12		**25.00**	**10.00**	**65.00**	**3.51**
M13		**25.00**	**27.50**	**47.50**	**3.84**
M14		**20.00**	**12.50**	**67.50**	**3.40**
Control		**0.0**	**0.0**	**100**	**0.0**
$LSD_{0.05}$		**12.93**	**6.87**	**-**	**-**

Table 2. Preliminary test for efficacy of *Trichoderma* spp. against *M. phaseolina in vitro.*

***Trichoderma* isolates**	**Bio-agents No.**	**Colony diameter of *M. phaseolina***	**Inhibition of *M. phaseolina* growth (%)**
T. harzianum (1)	T1	3.94	56.22
T. harzianum (2)	T2	4.42	50.88
T. harzianum (3)	T3	4.08	54.66
T. harzianum (4)	T4	4.13	54.11
T. harzianum (5)	T5	3.57	60.33
T. harzianum (6)	T6	3.92	56.44
T. harzianum (7)	T7	4.83	46.33
T. harzianum (8)	T8	3.40	62.13
T. harzianum (9)	T9	3.74	58.44
Average of *T. harzianum* isolates		**4.00**	**55.50**
T. hamatum (1)	T10	3.84	57.24
T. hamatum (2)	T11	4.06	54.88
T. hamatum (3)	T12	3.48	61.33
T. hamatum (4)	T13	4.28	52.35
T. hamatum (5)	T14	4.42	50.88
T. hamatum (6)	T15	4.72	47.55
T. hamatum (7)	T16	4.24	52.88
Average of *T. hamatum* isolates		**4.15**	**53.87**
T. viride (1)	T17	4.12	54.22
T. viride (2)	T18	4.06	54.84
T. viride (3)	T19	4.56	49.31

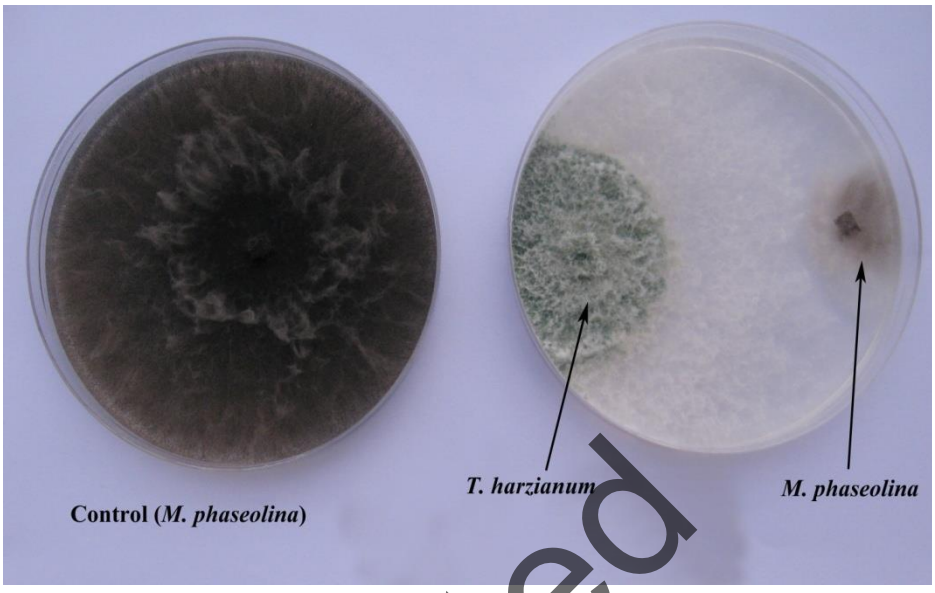
T. viride (4)	T20	4.10	54.44
Average of *T. viride* isolates		**4.21**	**53.20**
T. koningii (1)	T21	4.74	47.31
T. koningii (2)	T22	5.06	43.77
T. koningii (3)	T23	5.10	43.28
T. koningii (4)	T24	4.56	49.33
T. koningii (5)	T25	5.34	40.62
T. koningii (6)	T26	5.18	42.42
Average of *T. koningii* isolates		**4.99**	**44.45**
Control		9.00	-
$LSD_{0.05}$		0.76	-

Table 3. Bio-control efficacy of *Trichoderma* isolates against *sunflower charcoal rot* under greenhouse conditions (average of two successive experiments).

Treatments/Application time	Damping-off (%)		Survival plants %	Charcoal rot severity	Reductions in charcoal rot severity (%)
	Pre-emergence	Post-emergence			
T. harzianum 7 days before application of *M. phaseolina*	**0.0**	**17.50**	**82.50**	**2.47**	**38.40**
T. harzianum concurrently with *M. phaseolina*	**7.50**	**20.00**	**72.50**	**2.85**	**28.80**
T. harzianum 7 days after application of *M. phaseolina*	**20.00**	**17.5**	**62.50**	**3.05**	**23.81**
Average	**9.16**	**18.33**	**72.50**	**2.79**	**30.33**
T. hamatum 7 days before application of *M. phaseolina*	**2.50**	**25.00**	**72.50**	**2.92**	**27.18**
T. hamatum concurrently with *M. phaseolina*	**17.50**	**12.50**	**70.00**	**2.99**	**25.24**
T. hamatum 7 days after application of *M. phaseolina*	**25.00**	**12.50**	**62.50**	**3.20**	**20.07**
Average	**15.00**	**16.66**	**68.33**	**3.04**	**24.16**
M. phaseolina (infected control)	**42.50**	**17.50**	**40.00**	**4.01**	**0.0**
Uninfected control	**0.0**	**0.0**	**100**	**0.95**	**0.0**
$LSD_{0.05}$	**6.81**	**4.73**	**-**	**-**	**-**

Figure 1. Efficacy of *Trichoderma* spp. against *M. phaseolina in vitro*

***T. harzianum* (T8)**



***T. hamatum* (T12)**

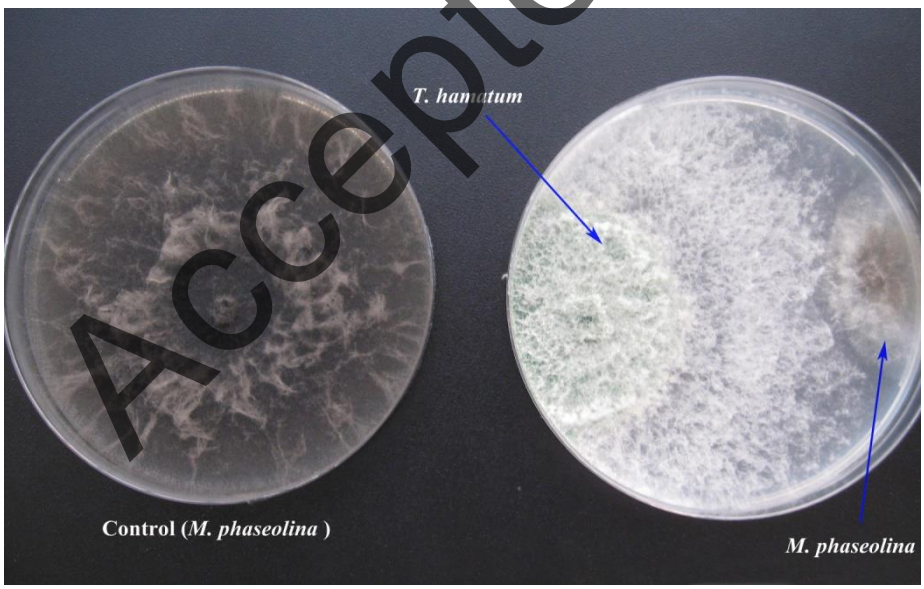
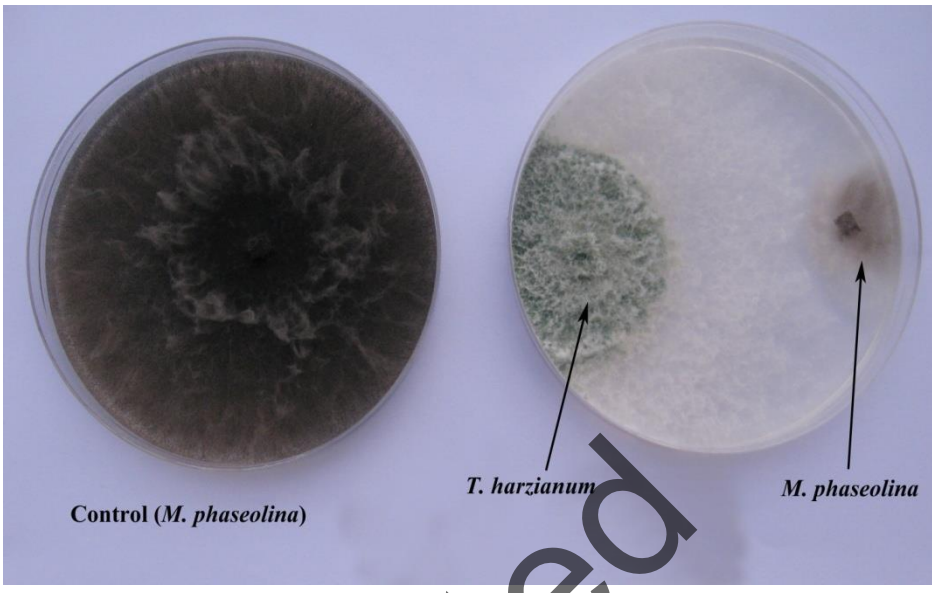


Figure 1. Efficacy of *Trichoderma* spp. against *M. phaseolina* in *vitro*

***T. harzianum* (T8)**



***T. hamatum* (T12)**