Egyptian Journal of Crop Protection, 14(1): 1-10.2019

BIO-CONTROL EFFICACY OF TRICHODERMA AGAINST STRAWBERRY CHARCOAL ROT DISEASE

El-Said Zaki Khalifa, Gomaa Abdel-Aleem Amer, Ramadan Abdelmoneim Bakr* and Abdullah Sobhy Hamad

Agricultural Botany Department, Faculty of Agriculture, Menoufia University, Egypt

ABSTRACT

Strawberry charcoal rot caused by *Macrophomina phaseolina* classified as one of the most destructive diseases. The current work was conducted to screen the efficacy of eight different Trichoderma isolates against two isolates of *M. phaseolina* under laboratory and greenhouse conditions. Dual culture technique results showed that the highest inhibitory rate of *M. phaseolina* isolates (M1 and M3) recorded with *T. viride*$_{(2)}$, whereas the least inhibitory effect recorded with *T. harzianum* under laboratory conditions. Greenhouse results showed that *T. hamatum* (1, 2) showed the highest percentage of survival plants infected with *M. phaseolina* isolates (1 and 3) while the lowest percentage of survival plants obtained with *T. harzianum*. Thus, Trichoderma can used for the control of strawberry charcoal rot as a biological control method and included in the organic production system.

Key words: Charcoal rot, Strawberry, *Macrophomina phaseolina*, Bio-control Trichoderma.

INTRODUCTION

In Egypt, Strawberry (*Fragaria ananassa* Duch.) considered an important economic export crop. It belongs to family Rosaceae and grown in protected houses and open fields. Egyptian production and exportation change during the last five years. Egypt was the first Arabian country in production of strawberry (AOAD, 2017). Strawberry cultivation distributed in different soil types in three main governorates in Egypt i.e., El-Behira, Qalubia and Ismailia. Strawberry plantation and production can be limited by several considerable soil-borne diseases (Porras *et al*., 2007). In 2014 the estimated losses in commercial strawberry production yield caused by fungal and nematodes diseases was 20-30 % (Conti *et al*., 2014). Recently strawberry charcoal rot disease caused by *M. phaseolina* (Tassi) considered an economically significant challenge and limitation factor in the regions of strawberry production worldwide (Chamorro *et al*., 2016). *Macrophomina phaseolina* is a wide spread soil borne pathogen with a wide host range of >500 plant species as it recorded in plants considered non susceptible previously (Kaur *et al*., 2012). The optimum conditions for the disease development is high temperature, shortage in soil water supply, sandy

*Corresponding Author E-Mail:ramadanbaker82@yahoo.com

soil and stressed plants (Mihail, 1989). Survival and longevity of *M. phaseolina* microsclerotia and the infected plant debris can help in consequently infection of strawberry plants season after season and increased the challenges in charcoal rot control programme (Dhingra and Sinclair, 1975). Recent management strategies are based on one or more from planting in free infested soil, soil solarisation and fumigation, using of resistance cultivars, transplant healthy pathogen free-plants, organic matter and compost (Gupta *et al*., 2012, Koike and Bolda 2013; Koike and Gordon, 2015).

Nowadays, thinking about replace the chemical fungicides with Bio-control agents are urgently needed because of the hazardous on the environment and emergence of fungicide-resistant. Microbial populations in the soil represent an important key role in phytopathogens suppression and promotion of plant health especially the beneficial microbes (Burkett-Cadena *et al*., 2008). Until now, there is different of friendly microbes in the soil with antagonistic ability un-known and survey needed. Soil in tropical country is a wealthy source with bio-control agents such as bacteria and fungi, which can use in biological control. Genus Trichoderma is one of the most spread imperfect fungi globally and widely isolated from various sources such as, agricultural soil, decaying woods and plant matters (Verma *et al*., 2007). In the last decades genus *Trichoderma* recorded as a promising bio-control agents which contain wide number of species (Wu *et al*., 2017). Previous investigation confirmed the pioneer effect of different *Trichoderma* species in control of various plant pathogenic fungi including *M. phaseolina* in different crops and enhancing the plant growth parameters (Khaledi and Taheri, 2016; Pastrana *et al*., 2016; Abdelatief and Bakr, 2018).

Therefore, our study aim to screen the ability of different Trichoderma isolates against the strawberry charcoal rot pathogen *M. phaseolina in vitro* and *in vivo* under Egyptian conditions.

MATERIALS AND METHODS

Source of the causal organism

Macrophomina phaseolina isolates were isolated from naturally infected strawberry plants showing charcoal rot symptoms collected from different strawberry growing areas in Beheira governorate as described by Bakr and Hamad(2019), then isolates grown and maintained on the potato dextrose agar medium (PDA) at 25 ± 1°C.

Isolation of Trichoderma Isolates

Isolation of the bio-control agent *Trichoderma* spp. was done using the dilution plate method. Soil samples were taken from the rhizosphere zone of strawberry plants by uprooting the infected plants with great care to obtain most of the intact root system and put in plastic bags, labeled with needed information and transferred to laboratory in ice boxes. The root systems were shaken gently to get rid

of most of the adhering soil particles. One gram of root system adherent soil particles was transferred to a wide mouth bottle containing 99 ml sterile distilled water. The bottles were shaken thoroughly or mechanically for 15 minutes. This gave an approximate dilution of 1/100. Serial dilutions were made according to Beninashemi and Dezeeuw (1969). Using pipette 500 µL of 10^5 dilution was transfer to Petri dishes contain Trichoderma Selective Medium (TSM) for *Trichoderma* species isolation (Elad *et al.,* 1982).The growing fungal colonies purified using the single spore and hyphal tip-technique, Then transferred into PDA slants.

Identification of Trichoderma Isolates:

Trichoderma Isolates were identified at the Agricultural Botany Department, Faculty of Agriculture, Menoufia University microscopically according to the descriptions adopted by Rifai (1969) and (Gams and Bisset, 1998).The pure cultures were stored at 4°C in the refrigerator for further studies.

***In-vitro* experiment:**

Two isolates of *M. phaseolina* (M_1 and M_3) and eight bio-control agents, i.e.,*Trichoderma viride* $_{(1)}$, *T. viride* $_{(2)}$, *T. viride* $_{(3)}$, *T. hamatum* $_{(1)}$, *T. hamatum* $_{(2)}$, *T. hamatum* $_{(3)}$, *T. Koningii* and *T. harzianum* were carried out in dual cultures under laboratory conditions. Petri dishes (9 cm in diameter) containing PDA medium were used in these trials. Different plates were inoculated with 0.5cm in diameter disks of the pathogen isolates obtained from 4 days old cultures. Each pathogen isolate was inoculated at one side of the plate and the opposite side inoculated with a disk of 0.5 cm in diameter of Trichoderma isolates, obtained from 3 days old culture. Three plates were used for each particular treatment as replicates. Three plates were inoculated with *M. phaseolina* (M_1 and M_3) only as a control treatment. Plates were incubated at 27°C. When the mycelial growth covered the enter media surface in control treatment, all plates were tested for:

Hyper-parasitism: the parasitism of a parasite on another one (The bio agent usually parasitizes the hyphae of the pathogen).

Antagonism: the bio-control agent partly or completely inhibits the growth of the pathogen or kills it.

Growth inhibition: the bio-control agent inhibits the pathogen growth.

Over growth: the hyphae of the bio-control agent growth faster, extensive and cover the hyphae of the pathogen when they grow in dual culture.

The percentage of growth reduction that pooled out using the following formula:

$$\% \text{ Reduction} = \frac{\text{Control} - \text{Treatment}}{\text{Control}}$$

Greenhouse experiment:

The efficacy of the different eight Trichoderma isolates were evaluated against two isolates of *M. phaseolina* (M_1 and M_3) under greenhouse

conditions. The inoculum of pathogenic isolates and bio-control agent were prepared using barley grain + corn meal and wheat bran medium. This medium was prepared into polypropylene bags (each contained 100g corn meal + 100g wheat bran + 100ml water), and then bags were autoclaved for 20 minute at 121°C then, bags inoculated with the tested Trichoderma and *M. phaseolina* isolates and incubated at 27°C for 15 days. Sterilized plastic pots (25cm in diameter) were filled with sterilized loamy soil and inocula of the bio-agents (3% of soil weight) were individually mixed thoroughly with the soil, then watered and left for 7 days under greenhouse conditions. Two days before sowing date pots contained bio-agent were inoculated with *M. phaseolina* isolates at the rate of 3% of soil weight. Two strawberry seedlings (Cv. Festival) were transplanted in each pot. Pots containing sterilized soil inoculated with *M. phaseolina* isolates only were used as control. Five replicates were used for each particular treatment. Disease incidence was recorded as percentages of plants initially show signs of water stress and subsequently collapse and number of survived plants (thirty days after transplanting).

Statistical analysis

Data were statistically analyzed by using analysis of variance and comparisons of means at the 5% level of significance according to the Duncan's multiple range test. The analysis performed with the software costat 6.3-version program.

RESULTS

***In vitro*:**

The effect of different *Trichoderma* species *i.e. T. viride*$_{(1)}$, *T. viride*$_{(2)}$, *T. viride*$_{(3)}$, *T. hamatum*$_{(1)}$, *T. hamatum*$_{(2)}$, *T. hamatum*$_{(3)}$, *T. Koningii* and *T. harzianum* on radial growth of *M. phaseolina* isolates was studied in dual culture in Petri dishes. Data indicated that all the tested *Trichoderma* spp. with different isolates reduced the growth of *M. phaseolina*, some tested isolates of Trichoderma grew over *M. phaseolina,* while others showed inhibition zones.

Results presented in table (1) and fig (1) clear that all the tested *Trichoderma* spp. reduced the growth of *M. phaseolina* (M1). *Trichoderma viride*$_{(2)}$ gave the highest growth reduction of *M. phaseolina*(M1) by 77% followed by *T. hamatum*$_{(1)}$ with 73%. On the other hand, *T. harzianum* gave the lowest growth reduction (43%). *Trichoderma harzianum, T. hamatum* and *T. Koningii* grew over the growth of *M. phaseolina*(M1). The highest over growth was observed with *T. Koningii* by 33 mm over *M. phaseolina*(M1) followed by *T. hamatum*$_{(1)}$ by14 mm. The Inhibition zone was observed between the growth of *M. phaseolina*(M1) and the three tested isolate of *T. viride*. The biggest Inhibition zone was observed with *T. viride*$_{(1)}$ by 3.6 mm followed with *T. viride* (3) by 3.0 mm.

Data Illustrated in table (2) and shown in fig (2) show the efficacy of the tested *Trichoderma spp*. against *M. phaseolina*(M3). *Trichoderma*

hamatum$_{(1)}$ and *T.* viride$_{(3)}$ showed the highest growth reduction of *M. phaseolina*(M3) by 71% followed *T. viride*$_{(1)}$ by 68%.While the lowest growth reduction was observed with *T. harzianum* by 45%. *Trichoderma harzianum, T. hamatum* and *T. koningii* grew over the growth of *M. phaseolina*(M3).The highest over growth was found with *T. koningii* by 31 mm followed by *T. hamatum*$_{(1)}$ by 21.3 mm. Also, inhibition zones were recorded between *M. phaseolina*(M3) and *T. viride* tested isolates. The highest inhibition zone was observed between *T. viride*$_{(1)}$ and *M. Phaseolina*(M3) by (5.6mm), while the smallest zone (1.6mm) recorded with of *T. viride*$_{(3)}$, while other tested Trichoderma isolates doesn't showed any inhibition zone.

Table (1): Effect of some *Trichoderma* spp. on growth of *M. phaseolina* (M1) under laboratory conditions.

	Linear growth (mm)	Growth reduction (%)	Bio action (mm)	
			Over Growth	Inhibition zone
***T. viride*$_{(1)}$**	32.00^{bc}	64.44	-	3.6
T. harzianum	51.00^{a}	43.44	6.3	-
***T. hamatum*$_{(1)}$**	24.00^{c}	73.33	14.0	-
***T. viride*$_{(2)}$**	20.66^{bc}	77.04	-	2.3
***T. viride*$_{(3)}$**	32.33^{bc}	64.07	-	3.0
***T. hamatum*$_{(2)}$**	30.00^{bc}	66.66	10	-
T. koningii	33.66^{b}	62.60	33.6	-
***T.hamatum*$_{(3)}$**	27.33^{bc}	69.63	8.6	-
Control(M1)	90.00	-	-	-
L.D.S	6.09	-	-	-

Columns followed by different letters are significantly different according to Duncan's Multiple Test ($p \leq 0.05$).

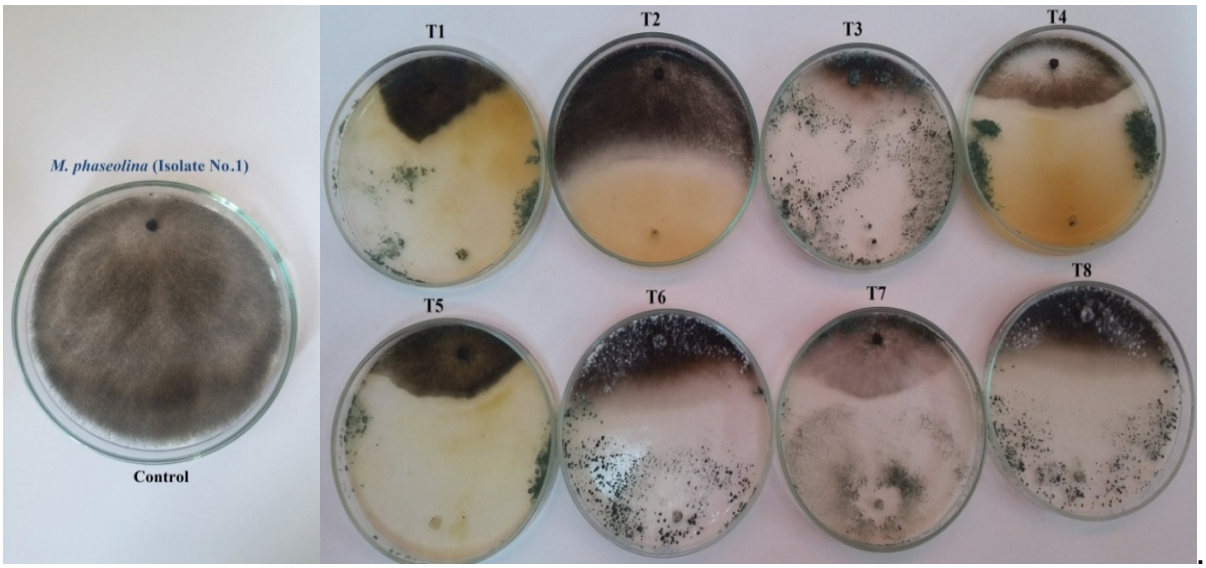

Fig.(1): Mode of action of eight *Trichoderma* spp. Isolates against *M. phaseolina* (M1). As T1, T4&T5 =*T. viride*, T2=*T. harzianum, T*3, T6&T8=*T. hamatum* and T7= *T. koningii.*

Table (2): Effect of some *Trichoderma* spp. on growth of *M. phaseolina* (M3) under laboratory conditions.

	Linear growth (mm)	Growth reduction (%)	Bio action (mm)	
			Over Growth	Inhibition zone
***T. viride*$_{(1)}$**	28.33^{b}	68.52	-	5.6
T. harzianum	48.66^{a}	45.93	5.0	-
***T. hamatum*$_{(1)}$**	25.66^{b}	71.48	21.3	-
***T. viride*$_{(2)}$**	32.33^{b}	64.07	-	2.3
***T. viride*$_{(3)}$**	26.00^{b}	71.11	-	1.6
***T. hamatum*$_{(2)}$**	29.33^{b}	67.41	12.0	-
T. koningii	31.66^{b}	64.82	31.6	-
***T.hamatum*$_{(3)}$**	26.33^{b}	70.74	12.0	-
Control(M3)	90.00	-	-	-
L.D.S	4.29	-	-	-

Columns followed by different letters are significantly different according to Duncan's Multiple Test ($p \leq 0.05$).

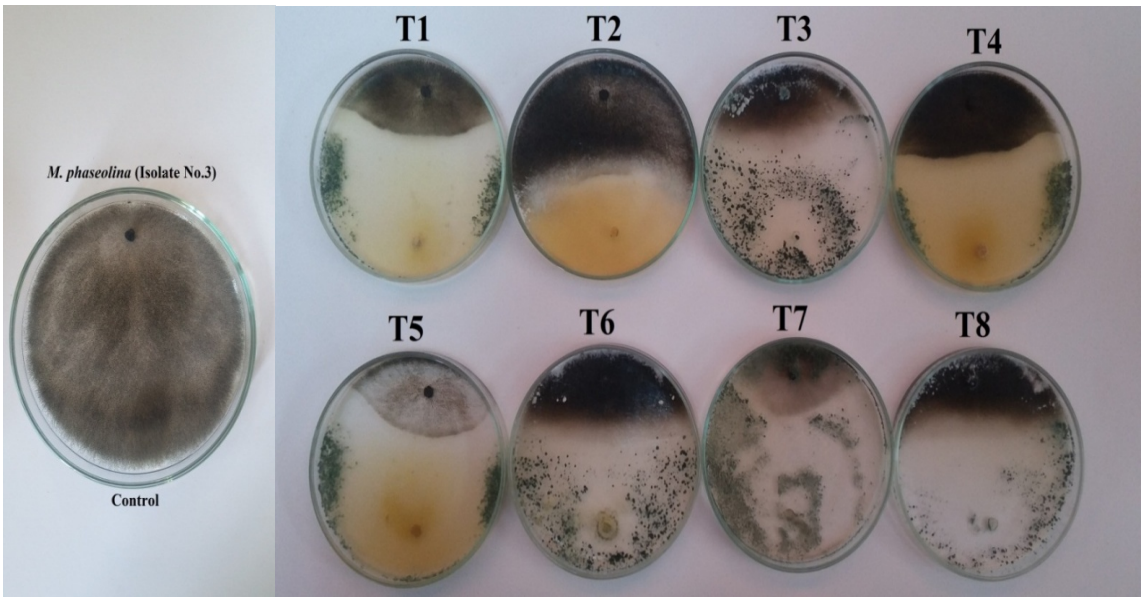

Fig. (2) Mode of action of eight *Trichoderma* spp. Isolates against *M. phaseolina* (M3). As T1, T4&T5 =*T. viride*, T2=*T. harzianum, T*3, T6&T8=*T. hamatum* and T7= *T. Koningii.*

Under greenhouse conditions:

Eight isolates representing four species of the tested biocontrol agent *Trichoderma* spp., i.e., *T. harzianum*, *T. hamatum*, *T. viride*, and *T. koningii* were tested as soil treatment for their effects against two virulent isolates of *M. phaseolina*, ie. M1 and M3.The bio-agents were tested against charcoal rot disease incidence on the susceptible strawberry cultivar "Festival".

Data in table (3) and fig (3) indicate that the initial symptoms of charcoal rot of strawberry caused by isolate (1) of *M. phaseolina* were decreased by

T. viride $_{(1)}$ and *T. hamatum*$_{(1)}$, while the other isolates were not effective in reducing the initial symptoms. All the tested isolates of Trichoderma decreased greatly the collapsed plants.*Trichoderma hamatum* $_{(2)}$ and $_{(3)}$ were the most effective ones by recording the lowest percentage of collapsed plants (10%) followed by *T. hamatum* $_{(1)}$,*T. viride* $_{(3)}$ and *T. koningii* (20%), while *T. harzianum* was the least effective one giving 50% collapsed plants comparing to the control (60% collapsed plants). *Trichoderma hamatum* $_{(1)}$ and $_{(2)}$ gave the lowest percentage of infected plants (30%), while *T. harzianum* record the highest infected plants (70%) comparing to the control treatment (80%). *Trichoderma hamatum* $_{(1)}$ and $_{(2)}$ gave the highest percentage of survival plants (70%) whereas, *T. harzianum* gave the lowest percentage of survival plants (30%) comparing to the control treatment (20%). Results presented in table (4) and fig (4) clear that the initial symptoms of charcoal rot caused by isolate (3) of *M. phaseolina* were decreased by isolate $_{(2)}$ and $_{(3)}$ of *T. hamatum* and *T. koningii* (10%), followed by all the tested isolates of *T. viride* (20%) comparing to the control (30% initial symptoms). *T. hamatum*$_{(1)}$ was the most effective isolates in reducing collapsed plants (10%) followed by isolates $_{(1)}$, $_{(2)}$ and $_{(3)}$ of *T. viride* (20%) comparing the control (70% collapsed plant). *Trichoderma hamatum*$_{(1)}$ and *T. koningii* were the most effective tested isolates in reducing percentage of infected plants (30%), one the other hand *T. harzianum* was the least effective one (80%) comparing the control treatment (100% infected plants). *Trichoderma hamatum* $_{(1)}$ and *T. koningii* gave the highest percentage of survival plants (70%), while *T. harzianum* gave the lowest percentage of survival plants (20%) comparing to the control treatment (0% survival plants).

Table (3): Effect of some *Trichoderma* spp. on charcoal rot of strawberry caused by isolate (1) of *M. phaseolina* under greenhouse.

	Initial symptoms(%)	Collapsed plants (%)	Infected plants (%)	Survival Plant (%)
***T. viride*$_{(1)}$**	10	30	40	60
T. harzianum	20	50	70	30
***T. hamatum*$_{(1)}$**	10	20	30	70
***T. viride*$_{(2)}$**	20	30	50	50
***T. viride*$_{(3)}$**	20	20	40	60
***T. hamatum*$_{(2)}$**	20	10	30	70
T. koningii	30	20	50	50
***T.hamatum*$_{(3)}$**	30	10	40	60
Control	20	60	80	20

Table (4): Effect of some Trichoderma spp. on charcoal rot of strawberry caused by isolate (3) of *M. phaseolina* under greenhouse.

Bioagent	Initial symptoms(%)	Collapsed plants (%)	Infected plants (%)	Survival Plant (%)
***T. viride*$_{(1)}$**	20	20	40	60
T. harzianum	30	50	80	20
***T. hamatum*$_{(1)}$**	20	10	30	70
***T. viride*$_{(2)}$**	30	20	50	50
***T. viride*$_{(3)}$**	20	20	40	60
***T. hamatum*$_{(2)}$**	10	30	40	60
T. koningii	10	20	30	70
***T.hamatum*$_{(3)}$**	10	30	40	60
Control	30	70	100	0

DISCUSSION

According to the high losses in strawberry production due to charcoal rot, management of the causal fungus has been mainly based on using the chemical fungicides. Using of Trichoderma play a good role as bio-control agent especially that it is isolated from the naturally infested soils. Trichoderma are generally utilized in agriculture as bio-control operators as a result of their capacity to lessen the occurrence of ailment brought about by plant pathogenic growths, especially numerous basic soil borne pathogen (Papavizas,1985 and Dubey *et al.,* 2007).

In vitro dual technique Trichoderma different species significantly inhibit the mycelium radial growth of *M. phaseolina* and the effect was varying according to the tested species. Our study showed *T. viride* $_{(1)}$ and *T. hamatum* $_{(1)}$ have a better growth inhibition of *M. phaseolina* compared to *T. harzianum*. These results are similar with many previous researchers such as Ramezani (2001) who reported that *T. harzianum* significantly inhibited the growth of *M. phaseolina*. *Trichoderma viride* and *T. harzianum* had a greater inhibition on *M. phaseolina* than *T. hamatum*. Khaledi and Taheri (2016) recorded inhibition in growth of *M. phaseolina* by different *Trichoderma* spp.

The mode of action of Trichoderma against the fungal pathogens may be directly such as mycoparasitism or indirectly by production of antibiotics and enzymes, enhance of plant defence and resistance mechanisms, Rhizosphere modification, competing for nutrient and antibiosis(Benítez *et al*., 2004).The importance of direct and indirect mechanisms in the bio-control process varying according to target antagonized fungus, Trichoderma strain, host plant, and environmental conditions, including temperature, pH and nutrient availability. The antagonistic action potency of Trichoderma mainly refers to the excretion of cell wall–degrading enzymes like chitinase, proteases and glucanase which prohibit the development of many fungal pathogens (Kucuk and Kyvanc, 2008).The variability in antagonistic efficacy and inhibition potency of Trichoderma may be referring to differences in level of produced hydrolytic enzymes by the Trichoderma

species or isolation site (Cherkupally *et al*, 2017).

Future view is to improve the commercial formulation of Trichoderma as biopesticides and bio-fertilizer for plant disease control and plant growth enhancement. In conclusion, Trichoderma should be an important element of integrated management of strawberry charcoal rot disease through bio-control agents with the replacement of chemical fungicides via modern and natural biopesticides without troublesome the ecological equilibrium of environment.

REFERENCES

Abdel-lateif, K.S. and R.A. Bakr (2018). Internal transcribed spacers (ITS) based identification of Trichoderma isolates and biocontrol activity against *Macrophomina phaseolina*, *Aspergillus niger* and *Meloidogyne incognita*. African Journal of Microbiology Research. 12(30): 715-722.

AOAD (2017). Arab Organization for Agriculture Development, arab agricultural statistics year book. VOL. No. (37).

Bakr, R.A and A.S. Hamad (2019). Survey, Varietal Reaction and Chemical Control of Strawberry Charcoal Rot in Egypt. Pakistan journal of Phytopathology, 31(1):55-66.

Benitez, T., A. M. Rincon, M.C., Limon and A.C. Condon (2004). Biocontrol mechanisms of Trichoderma strains. International Microbiology. 7 (4): 249–260.

Beninashemi, Z. and D.J. Dezeeuw (1969). Two improved methods for selectivity isolating Fusarium oxsporum from soil and plant roots. Plant Disease Reporter. 35: 589.

Burkett-Cadena, M., N. Kokalis-Burelle, K. S. Lawrence, E. Van Santen and J. W. Kloepper (2008). Suppressiveness of root-knot nematodes mediated by rhizobacteria. Biological Control. 47(1): p. 55-59.

Chamorro, M., T. E. Seijo, J. C. Noling, B. De los Santos and N. A. Peres (2016). Efficacy of fumigant treatments and inoculum placement on control of *Macrophomina phaseolina* in strawberry beds. Crop protection. *90*:163-169.

Cherkupally, R., S. R.Kota, H. Amballa and B. N. Reddy (2017). In vitro antifungal potential of plant extracts against *Fusarium oxysporum*, Rhizoctonia solani and *Macrophomina phaseolina*. Annalls of Plant Science. *6*(9): 1676-1680.

Conti, S., G. Villari, S.Faugno, G. Melchionna, S. Somma and G. Caruso (2014). Effects of organic vs. conventional farming system on yield and quality of strawberry grown as an annual or biennial crop in southern Italy. Scientia Horticulturae. 180: 63-71.

Dhingra, O.D. and J.B.Sinclair (1975). Survival of *Macrophomina phaseolina* sclerotia in soil: effects of soil moisture, carbon: nitrogen ratios, carbon sources, and nitrogen concentrations. Phytopathology .65: 236–240.

Dubey, S.C., M.Suresh and B. Singh (2007). Evaluation of *Trichoderma* species against *Fusarium oxysporum* f.sp. *ciceris* for integrated management of chickpea wilt. Biological Control. 40: 118-127.

Elad, Y., A. Kalfon and I. Chet (1982). Control of *Rhizoctonia solani* in cotton by seed coating with Trichoderma spores. Plant and soil. 66:279-282.

Gams, W. and J. Bissett (1998). Morphology and identification of Trichoderma. Harman GE, Kubicek CP (eds) Trichoderma and Glicoladium. Tayler and Francis ,London.

Gupta, G. K., S. K. Sharma and R. Ramteke (2012). Biology, epidemiology and management of the pathogenic fungus *Macrophomina phaseolina* (Tassi) Goid with special reference to charcoal rot of soybean (*Glycine max* (L.) Merrill). Journal of Phytopathology. 160:167-180.

Kaur, S., G. S. Dhillon, S. K. Brar, G. E. Vallad, R. Chand and V. B. Chauhan (2012). Emerging phytopathogen *Macrophomina phaseolina*: biology, economic importance and current diagnostic trends. Critical Reviews in Microbiology. *38*(2), 136-151

Khaledi,N. and P. Taheri (2016). Biocontrol mechanisms of *Trichoderma harzianum* against soybean charcoal rot caused by *Macrophomina phaseolina*. Journal of Plant Protection Research. 56 (1):21-31.

Koike, S. and M. Bolda (2013). Charcoal rot of strawberry: Increasing problem in California (blog). University of California Extension. August 2, 2013. http://ucanr. edu/ blogs/ blogcore/ postdetail. Cfm.postnum=11043.

Koike, S. T. and T. R. Gordon (2015). Management of Fusarium wilt of strawberry. Crop Protection. 73:67-72.

Küçük, Ç. and M. Kývanç (2008). Mycoparasitism in the biological control of *Gibberella zeae* and *Aspergillus ustus* by *Trichoderma harzianum* strains. Journal of Agricultural Technology. 4(2), 49-55.

Mihail, J.D. (1989). *Macrophomina phaseolina*: spatio-temporal dynamics of inoculums and of disease in a highly susceptible crop. Phytopathology. 79, 848e855.

Papavizas, G.C. (1985) *Trichoderma* and *Gliocladium*: Biology, Ecology and potential for biocontrol. Annual Review of Phytopathology. 23: 23-54.

Pastrana, A. M., M. J. Basallote-Ureba, A. Aguado, A. K. D. I.Khalid and N. Capote (2016). Biological control of strawberry soil-borne pathogens *Macrophomina phaseolina* and *Fusarium solani*, using *Trichoderma asperellum* and Bacillus spp. Phytopathologia Mediterranea. 55(1), 109-120.

Porras, M., C. Barrau, F.T. Arroyo, B. Santos, C. Blanco and F. Romero (2007). Reduction of *Phytophthora cactorum* in strawberry fields by *Trichoderma spp*. and soil solarization. Plant Disease. 91(2), 142-146.

Ramezani, H. (2001). Biological Control of Root-Rot of Eggplant Caused by *Macrophomina phaseolina*. American-Eurasian Journal of Agricultural and Environmental Science.4: 218-220.

Rifai, M. A. (1969). A revision of the genus Trichoderma. Common wealth Mycological Institute Kew, Surrey England. Mycological papers pp.116-156.

Verma, M., Brar, S. K., Tyagi, R. D., Surampalli, R. Y., and Valero, J. R. (2007). Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. Biochemical Engineering Journal. 37(1): p. 1-20.

Wu, Q., R.Sun, M. Ni, J.Yu, Y. Li, C.Yu,K. Dou, J .Ren and J.Chen(2017). Identification of a novel fungus, *Trichoderma asperellum* GDFS1009, and comprehensive evaluation of its biocontrol efficacy. PLoS One, 12, e0179957.

Received: April 30, 2019.
Revised: June 20, 2019.
Accepted: June 25, 2019.