

The efficiency of some bioagent fungi in reduction of wheat seed decay and seedling damping-off disease with heavy metals interaction

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Abstract. Madhi QH, Abass MH, Matrood AAA. 2021. The efficiency of some bioagent fungi in reduction of wheat seed decay and seedling damping-off disease with heavy metals interaction. *Biodiversitas* 22: 3984-3993. Biological control is an ecofriendly efficient measurement for disease control and heavy metals reduction in soils. The use of bioagent fungi such as *Trichoderma koningii* and *Chaetomium globosum* reduced the negative effect of pathogenic fungi that cause seed decay and the seedlings damping off wheat alone or by interaction with the concentrations of lead or cadmium, which increases the germination percentage of wheat seeds and reducing seedling damping off. It also reduced the severity index of wheat with pathogenic fungi and reduced the negative effect of interaction between heavy metals and pathogenic fungi on the severity index of the wheat. Results showed that *T. koningii* and *C. globosum* reduced the effect of the interaction of *R. solani* with 200 mg/kg lead to 57.7 and 55.4%, respectively and *R. solani* and cadmium 3 mg/kg with 60 and 61.6%, respectively. *T. koningii* and *C. globosum* also reduced the effect of the interaction *F. solani* with lead 200 mg/kg to 45.4 and 48.5%, respectively and *F. solani* and cadmium 3 mg/kg to 46.8 and 52.5% respectively. The bioagent fungi also increased the fresh and dry weight of shoot and root system, *T. koningii* significantly increased the fresh and dry weight of shoot in the presence of *R. solani*. The results also indicated that there was a high significant difference in the use of *C. globosum* in increasing the fresh and dry weight of shoot and root system. *T. koningii* and *C. globosum* significantly reduced the effect of interaction between the pathogenic fungi and low concentrations of lead and cadmium leading to an increase in the fresh and dry weight of shoot and root system. They also increased the plant height in the presence of pathogenic fungi as well as reducing the negative effect of the interaction between heavy metals and pathogenic fungi in the height of wheat plants. No significant interaction was observed between the low concentrations of lead and cadmium and pathogenic fungi in the presence of bioagent fungi. The results exhibited that bioagent fungi can reduce the negative effect of interaction of pathogenic fungi with lead and cadmium on the total phenols content of wheat leaves, and no significant difference was recorded in the treatment of low concentrations with the pathogenic fungi. Results showed that bioagent fungi can reduce the negative effect of the interaction of pathogenic fungi with lead and cadmium on the total phenols content of wheat plant leaves. No significant differences were recorded in the treatment of low concentrations with the pathogenic fungi in the presence of bioagent fungi. The two bioagent fungi increased the concentration of chlorophyll a and b, total chlorophyll and carotenoids reduced anthocyanin in leaves, and increased chlorophyll stability index compared to the control treatment.

Keywords: Damping-off, fungi, heavy metals, pathogenicity, wheat

INTRODUCTION

Triticum aestivum L. is the universal cereal of the old world agriculture and the world's foremost consumed crop plant after rice and maize (FAOSTAT 2011). This crop is currently cultivated in more than seventy different countries around the world, with approximately 3864.4032 hectares of cultivated areas, with about 3052.939 million tons of harvested grains (FAO 2018), and Iraq is among these countries with a production of 1518471 tons with an area of 633.1116 hectares (CASO 2019). Global cereal production needs a significant increase in production in the coming decades to meet the food and feed consumption needs of humankind. Global production of wheat, corn, rice and soybeans peaked at 2,102 million tons of harvested grains in 2018. Pre-harvest losses due to diseases, animal pests, weeds, abiotic and harvest pressures annually destroy about 35% of total biological output and lead to direct and indirect impact of pests on production results in heavy losses in grains (Mesterhazy et al. 2020).

Abiotic stress, biological control of plant pests and pathogens, and bio-plant treatment have gained great importance in recent years due to climate change and soil and natural resource pollution that increase crop stress, limiting and reducing production (Bamisile et al. 2018; Nandy et al. 2020). The growth of plants is mainly supported by the microorganisms that live in the soil area near the roots i.e. the rhizosphere, which includes many life forms such as fungi, bacteria and viruses, and also inside the plant, i.e. the endosphere (Manara 2012). The microbes present in the rhizosphere are tolerant of minerals and facilitate plant growth through several mechanisms through the proliferation of root filaments, thus increasing the surface area, and facilitating mineral absorption and directing to the aerial parts of plants (Wu et al. 2009). Pischik et al. (2009) stated that microbes are found in both the endosphere and the rhizosphere, and aid in the absorption, dissolution and tolerance of heavy metals (Zhu et al. 2014). Endophytes are those that can be isolated from healthy plant tissues including beneficial neutral or

commensal microorganisms, as well as inactive microorganisms and underlying pathogens. It is well known that some endophytes contribute to plant health and aid in plant adaptation. Biotic and abiotic stress conditions (Mani and Natesan 2018). Some endophytic fungi such as *Chaetomium globosum* are able to reduce the damage caused by plant pathogens by their hostility by excessive parasitism, competition, or production of antibiotics, or by activating plant defenses. Endogenous fungi are able to increase plant tolerance to abiotic stress such as dehydration or salinity or elevation of temperature and heavy metals by activating plant stress responses, allowing plants to avoid or mitigate the effect of stress (Fu et al. 2007; Siya et al. 2020). *Trichoderma* sp. is commonly found in roots, soil, and all environmental conditions. It can grow faster and produce strong spores and enzymes that degrade pathogen cell walls. It is an environmentally friendly biological control agent and is characterized by high resistance to various toxins and biotic compounds including antibiotics, fungicides and heavy metals (Harman et al. 2004). It has good produced antibody ability against some fungi that cause plant diseases (Benitez et al. 2004). Kredics et al. (2001) reported that mercury (Hg) does not affect the production of extracellular enzymes of *T. harzianum*. For this reason, it is more effective in the case of heavy metal stress against the fungal pathogens. The aim of the present research is to find out the effect of biological fungi on seed pathogen interference, seed death, lead, and cadmium concentrations.

MATERIALS AND METHODS

The experiment was carried out in the Laboratories of the Plant Protection Department, and the field experiment was carried out at the Agricultural Research Station of the College of Agriculture - University of Basrah.

Sources of fungal isolates

The isolates of pathogenic fungi *Rhizoctonia solani*, *Fusarium solani* and *Macrophomina phaseolina* were isolated from the root and soil of wheat plant according to Abass et al. (2021). In addition, the two fungi *Trichoderma koningii* and *Chaetomium globosum* were obtained from the Laboratories of the Prevention Department/College of Agriculture/University of Basrah.

Field experiment

Soil was collected from the Kumet area in the Maysan governorate, and the analysis of lead and cadmium in the soil showed that these minerals did not exceed the permissible standard limits for heavy metals EU (2006) also the concentrations of lead and cadmium were determined based on Madhi et al. (2020). Three concentrations of each element were determined for the study treatments, as cadmium chloride CdCl₂ was used as a source of cadmium and lead acetate Pb(C₂H₃O₂)₂ as a source of lead, and 200, 400, 600 mg/kg concentrations were used for lead and 3, 6 and 10 mg/kg for cadmium. The sterilized soil was distributed in plastic pots of 5 kg

capacity and in equal quantities. The inoculum of each pathogenic fungi and bioagent fungi was applied on the seeds of local millet (treatment) at a rate of 2% (weight/weight) (Jones et al. 1984). Then the seeds of wheat cultivar Iba 99 were planted and 10 seeds were sown per pot. Sterile water was used as irrigation water and with equivalent field capacity and the specifications of the water were (pH 7.5 and electrical conductivity 1.3 Desmens/m). No significant concentration of elements were determined in the water samples. Treatments that include the addition of heavy metals were watered at all three concentrations and the experiment lasted for 60 days. The experiment was carried out using a complete randomized design (CRD) with three replicates per treatment:

1. control
2. R.s+T.gon
3. R.s+C.glob
4. F.s+T.con
5. F.s.+C.glob
6. M.ph+T.con
7. M.ph+C.glob
8. R.s+T.con+ Pb200
9. R.s+T.con+ Pb600
10. R.s+T.con+ Cd3
11. R.s+T.con+ Cd10
12. R.s+C.glob + Pb200
13. R.s+C.glob + Pb600
14. R.s+C.glob + Cd3
15. R.s+C.glob +Cd10
16. F.s+T.con+ Pb200
17. F.s+T.con+ Pb600
18. F.s+T.con+ Cd3
19. F.s+T.con+ Cd10
21. F.s+ C.glob+ Pb600
22. F.s+ C.glob+ Cd3
23. F.s+ C.glob+ Cd10
24. M.ph+T.con+Pb600
25. M.ph+T.con+ Pb200
26. M.ph+T.con+ Cd3
27. M.ph+T.con+ Cd10
28. M.ph+ C.glob+ Pb200
29. M.ph+ C.glob+ Pb600
30. M.ph+ C.glob+ Cd3
31. M.ph+C.glob.+ Cd10

The experiment lasted for sixty days, and after the end of the experiment the following measurements were taken:

The percentage of germination and damping-off :

$$\text{Germination\%} = \frac{\text{Number of geminated seeds}}{\text{Total number of seeds}} \times 100$$

$$\text{Seedling damping - off\%} = \frac{\text{Number of damping - off seedling}}{\text{Total number of seedlings}} \times 100$$

Severity Index: The severity of the infection was calculated according to the scale mentioned by Wheeler (1970) consisting of four degrees as follows: (0) Seedlings are intact, (1) Seedlings are alive but infected with root rot ,

(2) pre-emergence damping off, (3) post-emergence damping off

The severity index was calculated according to the following equation:

$$\text{Severity Index \%} = \frac{\text{Total of seedling} \times \text{degree}}{\text{Total of seedlings} \times \text{higher degree}} \times 100$$

Average plant height (cm): The height of the plant was measured by a tape measure from the soil surface to the top of the plant.

Fresh and dry weight of the shoot and root (gm): The fresh weight was calculated by taking three plants for each treatment by cutting the plants at their contact area with the soil then the plants were dried using an electric oven at a degree of 60 m for 72 hours. Then the dry weight was calculated using the sensitive balance.

Determination of total phenols: Phenol contents was determined in wheat leaves by following the Singleton and Rossi method (1965). Briefly, 200 mg of leaves were weighed and added 2 ml of 50% methanol and placed in a mechanical shaker at a speed of 200 rpm/minute at room temperature for two hours. Then the filtrate was separated by a centrifugation process at a speed of 10,000 rpm for 15 minutes. The filtrate was kept in a 4 ml vial and the extraction process was repeated with the same previous steps. The second filtrate was mixed with the first filtrate. 200 microliters of filtrate were taken in test tubes after that 1.5 ml of Folin-Ciocalten reagent was added (diluted to ten times with distilled water) and left for five minutes at room temperature, then add 1.2 ml sodium bicarbonate Na_2CO_3 (7.5%) (weight/volume) and left the mixture for 60 minutes before reading the absorbance along with the length 765 nm. A sample was prepared with the same steps but without adding a filtrate as a control sample. The total amount of phenols was calculated using the standard Gallic acid curve. The results were expressed as equivalent to Gallic acid in mg/g fresh weight unit.

Photosynthetic pigments and chlorophyll stability index: Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids and anthocyanin were estimated according to Arnon method (1949). 200 mg of leaves were crushed in 8 ml of acetone (80%) in a chilled ceramic mortar, followed by centrifugation at 3,000 rpm for 30 min. The optical density of chlorophyll-a, chlorophyll-b, carotenoids and anthocyanin were recorded at 645, 663, 534, 470 nm, respectively. Acetone was used as a control sample to calibrate the instrument, use a spectrophotometer model UV-1100D. The pigments were estimated according to the following equations Asare-Boamah et al.(1986) and expressed in (mg/g).

$$\text{Chlorophyll - a} = 12.7 (\text{OD}663) - 2.69 (\text{OD}645) \times \text{Vol./Wt}$$

$$\text{Chlorophyll - b} = 22.9 (\text{OD}645) - 4.68 (\text{OD}663) \times \text{Vol./Wt}$$

$$\text{Total chlorophyll} = 20.2 (\text{OD} 645) + 8.02 (\text{OD} 663) \times \text{Vol./ Wt.}$$

$$\text{Anthocyanins} = 0.0821 \times \text{A}534 - 0.0439 \text{A}643 - 0.002423 \times \text{A}661$$

$$\text{Carotenoids} = \text{A}470 - 17.1 \times (\text{Chl - a} + \text{Chl - b}) - 9.479 \times \text{anthocyanins} / 119.26$$

Chlorophyll stability index (CSI)

Chlorophyll stability index was calculated in stress and control plants by following formula (Sairam et al. 1997):

$$\text{CSI (\%)} = \frac{\text{Total Chlorophyll in control plant}}{\text{Total Chlorophyll in stressed plant}} \times 100$$

RESULTS AND DISCUSSION

The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction

The results indicated the significant effect of the bioagent fungi against the pathogenic fungi showing an increase in the percentage of wheat seed germination and a decrease in seedling damping off. The treatment of the bioagent fungus *T. koningii* and the pathogenic fungus *R. solani* recorded germination and seedling damping-off percentage of 73.33 and 27.05% respectively, and the percentage of germination and seedling damping-off due to treatment of fungus *T. koningii* and *F. solani* were recorded as 76.66 and 23.03%, respectively. The percentage of seed germination increased and damping-off decreased due to the presence of the biological fungus *C. globosum*, and the highest effect was recorded by the pathogen *M. phaseolina*, as the germination percentage increased to 80% and the damping-off decreased to 5.93%. There was less biological effect on the pathogen *R. solani*, which was recorded 76.66 and 26.06%, respectively, as compared to the control treatment (Table 1).

The results of Table 1 showed that the bioagent fungi reduced the negative effect of interaction between heavy metals and pathogenic fungi on the percentage of germination and seedling damping-off. *T. koningii* reduced the interaction effect between lead 200 mg/kg and *R. solani* to 70.00 and 27.06%, respectively, and also reduced the interaction between cadmium 3 mg/kg and *R. solani* with 66.66 and 25.23%, respectively as compared to the treatment. *C. globosum* reduced the effect of the interaction of *R. solani* and 200 mg/kg of lead by 73.33 and 27.85%, respectively, and the interaction of *R. solani* and 200 mg/kg of cadmium reduced it to 70 and 27.05%, respectively, compared with the interaction of biotic fungi and the pathogen without lead or cadmium. The reason for the biological ability of the fungi to increase the germination rate of wheat seed and reduce the seedling damping-off may be attributed to the excretion of *Trichoderma* sp. Several pathogens secrete cell wall degrading enzymes (CWDE), such as chitinases and β -1,3-glucanases, also inhibits the formation of sclerotia in *R. solani* (Naeimi et al. 2010). The fungus *C. globosum* produces enzymes such as xylanase and 1,3-glucanase and hydrolytic enzymes. It also produces various metabolites such as chaetomin and chaetoglobosin that have anti-fungal properties (Ahmed et al. 2012). *C. globosum* produce pectin degrading enzymes polygalacturonate trans-Eliminase (PGTE), pectin trans-eliminase (PTE), polygalacturonase (PG), pectin methylesterase (PME), protopectinase (PP), methylesterase (PME), protopectinase (PP), and protopectinase (PP). Cellulolytic and several bioactive substances such as chaetoglobosin A, Chaetomium B, C, D, Q, R, T,

chaetocin, chaetomin, chaetochalasin A, and chaetoviridins A and C was also produced by *C. globosum* (Ashwini 2019). Pandey et al. (2019) reported that mechanisms that biotic fungi use are antifungal production, specialized antigenicity, and stimulation of plant immunity. It can also be attributed to the role of biotic fungi in analyzing organic and inorganic pollutants in the soil, reducing the toxicity of pollutants to plants, providing a good environment for plant growth and forming a physical barrier around plant roots that protect them from unfavorable environmental conditions and limit the invasion of pathogens and pests (Wu and Lin 2003). Moubarak and Abdel-Monaim (2011) reported that *Trichoderma* sp. reduced seedling fall and wheat root rot caused by *R. solani* and *F. solani* fungi compared to the control treatment. Abbas et al. (2017) indicated that *Trichoderma* sp. inhibited the *R. solani* fungus that caused root rot and seedling damping-off pre and post-emergence by having many mechanisms such as parasitism, production of antibiotics and competition in addition to stimulating plant defense responses. Latz et al. (2018) reported that *C. globosum* protects the plant from disease, reduces infection levels, and inhibits pathogen growth.

The results of Table 1 indicated that biotic fungi *T. koningii* and *C. globosum* reduced the severity index of wheat with pathogenic fungi. *T. koningii* reduced the severity index of *R. solani*, *F. solani* and *M. phaseolina* to 51.23, 40.8 and 22.05%, respectively, also *C. globosum* reduced the severity of infection by 45, 42.2 and 15.3%. The results showed that the biological fungi reduced the negative effect of the interaction between heavy metals and pathogenic fungi on the severity of wheat plant infestation. *T. koningii* and *C. globosum* reduced the effect of *R. solani* interaction with lead 200 mg to 57.7 and 55.4%, respectively, and they also reduced the interaction effect between *R. solani* and cadmium 3 mg/kg to 60 and 61.6%, respectively, compared with the pathogen and the bioagent fungus without lead or cadmium. The reason for the reduced severity index may be attributed to the role of biotic fungi in protecting plants from pathogens and abiotic stresses such as high concentrations of heavy metals, as well as enhancing plant growth through biosynthesis of plant hormones and nutrients acquisition (Humberto et al. 2020). Azevedo and Araujo (2007) stated that biofungi stimulate plant metabolism changes that alter their response to environmental stress and pathogen attack.

In addition, this interaction leads to the production of secondary metabolites by both fungi and plants, which enhances the ability to respond to environmental stress. *Trichoderma* sp. it secretes various biological metabolites that are toxic to plant pathogenic fungi such as pyrones, koniginins, viridian, gliovirin, gliotoxin, peptaibols and others (Vinale et al. 2014). *C. globosum* produces several bioactive compounds such as azaphilones (Yamada et al. 2011) and armochaetoglobins A – J, which are antifungal, cytotoxic and inhibitory for pathogenic fungi (Chen et al.

2015). Kredics et al. (2001) reported the use of *Trichoderma* sp. against *R. solani* and *Fusarium* sp. in soils contaminated with heavy metals, it produced the main active parasitizing enzymes, such as chitinase, protease, or B-1,3 glucanase, which participate in the degradation of fungal cell walls. All strains of *Trichoderma* sp. showed the best tolerance levels for nickel and manganese. Demirici et al. (2011) reported that *Trichoderma* sp. produces various enzymes cutinase, cellulase, protease, and B-1,3-gluconase, and produces many antibiotics such as Trichodermin, Pachybas, and Emodin chrysophancol as well as the production of toxic substances such as Gliotoxin that inhibit the bacterium of fungi. The fungus *C. globosum* reduced the severity of wheat plant infestation with *Bipolaris sorokiniana* by producing many active compounds such as chaetoviridin A, chaetoviridin E, chaetoglobosin C, epichaetoviridin A, epichaetoviridin T and prochaetoglobosins I (Yue et al. 2018).

Table 1. The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction on wheat seed germination and seedling damping-off

Treatments	Percentage		
	Germination	Damping off	Severity index
Control	100.000	0.000	00.00
R.S. +T.gon	73.333	270.500	51.23
R.S. +T.gon+Pb200	70.000	270.600	57.7
R.S. +T.gon+Pb600	63.333	292.833	64
R.S. +T.gon+Cd3	666.667	252.333	60
R.S. +T.gon+Cd10	566.667	26.666	70.25
R.s. + C.glob	766.667	260.667	45
R.s. + C.glob+Pb200	733.333	278.500	55.4
R.s. + C.glob+Pb600	666.667	294.333	62.5
R.s. + C.glob+Cd3	70.000	270.500	61.6
R.s. + C.glob+Cd10	60.000	359.500	67.875
F.S. +T.gon	766.667	230.333	40.8
F.S. +T.gon+Pb200	766.667	254.333	45.4
F.S. +T.gon+Pb600	70.000	278.500	66
F.S. +T.gon+Cd3	733.333	254.333	46.8
F.S. +T.gon+Cd10	63.333	302.333	67.00
F.s. + C.glob	80.000	169.000	42.2
F.s. + C.glob+Pb200	80.000	254.333	48.5
F.s. + C.glob+Pb600	70.000	278.500	60
F.s. + C.glob+Cd3	76.667	246.667	52.5
F.s. + C.glob+Cd10	666.667	302.333	63.33
M.ph +T.gon	80.000	5.930	22.05
M.ph. +T.gon+Pb200	760.000	100.000	25
M.ph. +T.gon+Pb600	70.000	15.000	51.00
M.ph. +T.gon+Cd3	73.333	117.833	30.11
M.ph +T.gon+Cd10	70.000	255.333	60.5
M.ph.+ C.glob	83.333	111.867	15.3
M.ph. + C.glob+Pb200	80.000	152.333	24.4
M.ph + C.glob+Pb600	766.667	194.333	46.8
M.ph. + C.glob+Cd3	80.000	167.833	36.8
M.ph. + C.glob+Cd10	70.000	219.000	57.7
L.S.D	6.516	2.475	6.702

The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction on shoot and root growth of wheat plants

Results showed that bioagent fungi have the ability to increase the fresh and dry weight of the shoot and root system, as *T. koningii* recorded a significant increase in the fresh and dry weight of the shoot in the presence of *R. solani*, which was 5.792 and 2.790 gm, respectively, and a significant increase in the fresh and dry weight of the root system was 2.029 and 0.637 g, respectively (Table 2). The highest significant increase was recorded in the treatment of *T. koningii* in the presence of *M. phaseolina* with 6.538 and 3.921 g, respectively, and the fresh and dry weight of shoot was 3.000 and 1.144 g, respectively. The results also indicated high significant differences in the use of *C. globosum* in increasing the fresh and dry weight of the shoot and root system, and the highest significant effect against *R. solani* for the fresh and dry weight of the root system. The result of statistical analysis indicated that there was high significant differences between *C. globosum* and *M. phaseolina* in increasing in the fresh and dry weight of the shoots, which was 7.085 and 2.911 gm, respectively, and increase in the dry and fresh weight of root system was 3.142 and 1.199 gm, respectively, compared to the control treatment, which recorded a fresh and dry weight of the shoot system being 4.531 and 1.959 g, respectively, and fresh and dry weight of the root system was recorded as 3.055 and 1.222 gm, respectively. The results of the statistical analysis indicated that the use of *T. koningii* and *C. globosum* significantly reduced the effect of interaction between the pathogenic fungi and the low concentrations of lead and cadmium on increasing the fresh and dry weight of the vegetative and root system, and no significant differences were recorded for their interaction compared with the interaction treatments for the biofungal and pathogenic fungi.

The ability of bioagent fungi to increase the fresh and dry weight of shoot and root system may be attributed to the fact that biotic fungi help plants to overcome abiotic stress and enhance plant growth through biosynthesis of phytohormones such as Andol-3, Acetic acid, Acetic acids, Gibberellins, Cytokinins, Ethylene, Eston and 2 3- Putanidol as well as nutrient absorption (He et al. 2020), Yedidia et al. (2001) reported that *Trichoderma* sp. can increase the phosphorus content in plants and nutrients that increase the shoot and root systems. *Trichoderma* sp. is a soil-borne filamentous fungi widely used for its numerous plant health benefits, such as giving their hosts improved growth, disease resistance, and abiotic stress tolerance. Several *Trichoderma* species are able to produce phytohormone indole-3-acetic acid (IAA), its production has been suggested to promote root growth and increase the

production of chlorophyll content (Nieto-Jacobo et al. 2017).

The use of *T. koningii* against *F. oxysporium* increased the fresh and dry weight of bean seedlings, the fresh weight of root increased by 1.4 to 1.5 g and the dry weight of the root increased by 1.4 to 1.8 g (Otadoh et al. 2011). Singh et al. (2014) reported that the use of *Trichoderma* sp. against *R. solani* increased the fresh and dry weight of the rice plant seedlings. Inoculation of wheat with *C. globosum* resulted in a significant increase in plant growth and increased phosphorous content in plant tissues, increasing biomass and root length by 25% and 39%, respectively (Yan et al. 2019). Murphy et al. (2019) reported that endophyte fungi such as *C. globosum* stimulate the growth of wheat and barley plants and increase the plant biomass.

Result exhibited that bioagent fungi have the ability to increase plant height in the presence of pathogenic fungi. and *T. koningii* achieved a significant increase of 16.2 cm in the presence of *R. solani*. *C. globosum* recorded a significant increase of 17.2 cm in the presence of the *R. solani*, and *F. solani* came to second, which scored 18.4 cm and did not record any significant differences between them, but they differed significantly from *M. phaseolina*, which achieved 20.9 cm. The bioagent fungi reduced the negative effect of interaction between heavy metals and pathogenic fungi on wheat plant height. No significant effect was observed between the low concentrations of lead or cadmium and the pathogenic fungi in the presence of the biological fungi compared with the treatment of the biological and pathogenic fungi, and the interaction record of *R. solani* and *T. koningii* with 200 mg/kg of lead and 3 mg/kg of cadmium showed an increase of 15.2 and 14.9 cm in height, respectively, and *R. solani* and *T. koningii* with 200 mg/kg of lead and 3 mg/kg of cadmium increased the height by 16 and 15.5 cm, respectively (Table 2).

The role of bioagent fungi in increasing plant height can be attributed to enhancing plant growth by converting nutrients or producing plant hormones or providing iron to reduce the harmful effects of mineral pollution to plants (Rajkumar et al. 2010; Deng 2014). Some bioagent fungi can reduce heavy metal toxicity and improve plant growth. Wei et al. (2014) reported that mechanisms for heavy metal biogenic fungi carrying include extracellular deposition, cell wall binding, intracellular influx of heavy metal ions by intracellular linkages, cell division, and the protective role of antioxidant systems. Different types of species have been studied for the production of antibiotics, the ability to improve plant growth and development, and its efficiency as a biological control agent (Contreras-Cornejo et al. 2009). *Trichoderma* species have been used in the biological treatment of soil and water pollutants such as heavy metals, biological foreign substances, toxins and environmental pollutants (Siddiquee et al. 2015).

Table 2. The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction on shoot and root growth of wheat plants

Treatments	Fresh weight of shoot	Dry weight of shoot	Fresh weight of root	Dry weight of root	Height of plant
Control	4.531	1.959	3.055	1.222	28.1
R.S. +T.gon	5.792	2.790	2.029	0.637	16.2
R.S. +T.gon+Pb200	4.17	2.808	2.029	0.511	15.2
R.S. +T.gon+Pb600	3.711	1.658	1.051	0.341	11.4
R.S. +T.gon+Cd3	4.518	2.314	2.046	0.677	14.9
R.S. +T.gon+Cd10	3.122	1.431	1.611	0.300	10.1
R.s.+ C.glob	5.841	2.938	2.041	0.716	17.2
R.s. + C.glob+Pb200	4.629	2.818	2.088	635	16
R.s. + C.glob+Pb600	4.126	1.417	1.077	0.467	11.9
R.s. + C.glob+Cd3	5.111	2.800	2.069	0.627	15.5
R.s. + C.glob+Cd10	3.866	1.768	1.698	0.440	11.8
F.S. +T.gon	5.916	3.222	2.892	0.900	18.3
F.S. +T.gon+Pb200	5.061	3.093	2.431	0.841	15
F.S. +T.gon+Pb600	4.500	2.071	1.823	504	14.5
F.S. +T.gon+Cd3	5.129	3.117	2.141	0.723	17
F.S. +T.gon+Cd10	4.000	1.900	1.811	0.523	13.5
F.s.+ C.glob	5.914	3.397	2.922	1.001	18.4
F.s. + C.glob+Pb200	5.400	3.124	2.707	0.983	17.2
F.s. + C.glob+Pb600	4.222	1.674	1.199	0.563	15.3
F.s. + C.glob+Cd3	5.311	3.078	2.602	0.857	17.5
F.s. + C.glob+Cd10	4.146	1.916	1.487	0.439	14.8
M.ph +T.gon	6.538	3.921	3.000	1.144	20.3
M.ph. +T.gon+Pb200	6.376	3.900	2.981	1.000	15.5
M.ph. +T.gon+Pb600	5.145	2.866	1.976	0.911	16
M.ph. +T.gon+Cd3	6.000	3.715	2.509	0.900	18.3
M.ph +T.gon+Cd10	5.000	2.701	1.995	0.635	15
M.ph.+ C.glob	7.085	3.911	3.142	1.199	20.9
M.ph. + C.glob+Pb200	6.511	3.710	3.000	1.000	19.6
M.ph + C.glob+Pb600	5.31	2.977	2.400	0.844	17
M.ph. + C.glob+Cd3	6.467	3.699	1.087	0.944	18.7
M.ph. + C.glob+Cd10	5.157	2.843	1.012	0.740	16.5
L.S.D	1.289	0.393	0.508	0.292	2.351

The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction on some biochemical parameters of wheat plants

Results showed that bioagent fungi has the ability to reduce the negative effect of the pathogenic fungi interfering with lead and cadmium on the total phenols content of wheat leaves. No significant differences were recorded in the interaction treatments of low concentrations with pathogenic fungi. Bioagent fungi helped to reduce the negative effect of interaction between high concentrations and pathogenic fungi. The interaction of *T. koningii* with *R. solani* and lead (600 mg/kg) showed an increase in total phenols content by 9.67 mg/g. Also, the fungus *C. globosum* raised the total phenols content to 10.34 mg/g in the presence of 600 mg/kg lead. *T. koningii* and *C. globosum* also increased the total phenols content (9.22 and 9.87 mg/g) by their interaction between *R. solani* and 10 mg/kg of cadmium, which increased compared with the interaction treatment between *R. solani* and *T. koningii* without heavy metals which was recorded 15.45 mg/g as well as with the interaction treatment between *R. solani* and *C. globosum* without heavy metals was recorded 18.37 mg/g (Table 3). Phenols are aromatic benzene ring

compounds that contain one or more hydroxyl groups often produce by plants to protect against stress. It is difficult to overestimate the functions of phenolic compounds in plant physiology and interchange them with biotic and abiotic stress. Phenols are responsible for plant growth stages, especially in embryo biosynthesis and pigments. It also provides protection for plants, phenolic compounds secreted upon infection or in stress conditions that prevent or damage many microorganisms, and may combat some pathogens (Bhattacharya et al. 2010). The fungus *Trichoderma* sp. stimulates plant defenses in wheat through its by-products and the production of flavonoids, coumarins, and phenolic compounds that break the bonds of the active groups in trichothecenes (Moya 2010). Phenols are widely distributed in the plant and some occur mainly while others are synthesized in response to pathogen attack as their appearance is part of active defense (López-Bucio et al. 2015; Rao et al. 2015). *Trichoderma* sp. produces antioxidants and antimicrobials compounds in addition to their ability to enhance plant structural barriers that prevent disease spread (Ghassempour et al. 2011).

Table 3. The efficiency of bioagent fungi in reduction of seed fungal pathogens and heavy metals interaction on some biochemical parameters of wheat plant

Treatments	Total phenols	Chlorophyll a	Chlorophyll b	Total chlorophyll	Anthocyanins	Carotenoids	CSI
Control	26.475	30.833	18.404	16.372	1.06	9.273	100
R.S. +T.gon	15.456	13.64	6.391	5.822	2.266	5.867	35.560
R.S. +T.gon+Pb200	17.851	12.207	5.899	5.566	2.542	5.282	33.997
R.S. +T.gon+Pb600	9.676	9.524	3.244	4.429	2.721	3.259	27.052
R.S. +T.gon+Cd3	14.847	10.68	4.244	5.493	2.311	5.133	33.551
R.S. +T.gon+Cd10	9.222	7.37	2.433	3.923	2.931	3.351	23.961
R.s.+ C.glob	18.372	14.172	6.861	6.556	2.625	6.986	40.043
R.s. + C.glob+Pb200	17.084	13.54	6.600	6.533	2.466	6.623	39.903
R.s. + C.glob+Pb600	10.345	98.888	4.751	5.122	2.852	4.395	31.285
R.s. + C.glob+Cd3	16.976	12.239	6.000	6.484	2.000	5.604	39.604
R.s. + C.glob+Cd10	9.874	7.421	3.512	4.456	22.073	3.255	27.217
F.S. +T.gon	19.803	16.879	5.925	6.503	1.926	6.284	39.720
F.S. +T.gon+Pb200	17.101	15.175	5.000	6.301	2.246	5.737	38.486
F.S. +T.gon+Pb600	10.123	11.709	4.560	4.644	2.408	4.179	28.365
F.S. +T.gon+Cd3	14.314	14.161	5.167	5.755	2.111	5.125	35.151
F.S. +T.gon+Cd10	9.234	10.615	3.251	4.311	2.471	3.375	26.331
F.s.+ C.glob	22.003	17.913	6.121	7.654	1.728	6.544	46.759
F.s. + C.glob+Pb200	21.082	17.412	5.472	6.553	2.000	6.000	40.025
F.s. + C.glob+Pb600	14.375	13.305	5.222	4.636	2.712	4.568	28.316
F.s. + C.glob+Cd3	20.83	16.799	5.665	6.000	1.800	5.943	36.647
F.s. + C.glob+Cd10	13.196	12.517	4.232	4.121	1.488	4.349	25.171
M.ph +T.gon	24.865	22.471	8.888	7.151	1.878	6.927	43.678
M.ph. +T.gon+Pb200	23.817	21.711	8.71	7.000	2.522	6.368	42.755
M.ph. +T.gon+Pb600	18.983	16.461	7.088	6.565	1.898	3.825	40.098
M.ph. +T.gon+Cd3	22.815	21.36	8.919	6.988	2.000	4.154	42.682
M.ph +T.gon+Cd10	17.845	14.61	6.341	6.323	2.249	3.973	38.620
M.ph.+ C.glob	25.76	24.072	9.432	8.802	1.158	6.852	53.762
M.ph. +C.glob+Pb200	24.415	24.033	9.356	8.765	2.000	6.751	53.536
M.ph + C.glob+Pb600	18.783	19.276	8.111	7.232	2.846	5.239	44.172
M.ph. + C.glob+Cd3	23.783	23.333	9.473	8.345	2.137	6.461	50.971
M.ph. + C.glob+Cd10	19.339	18.563	8.000	7.225	2.209	4.244	44.130
L.S.D	2.163	1.548	1.533	1.583	1.379	1.910	2.348

A high level of chlorophyll was recorded in the leaves of wheat plant due to the use of *T. koningii* and *C. globosum*. *C. globosum* recorded the highest significant effect against *M. phaseolina*, and the content of chlorophyll a, b, total chlorophyll and carotenoids increased to 24.072, 9.432, 8.802 and 6.852 mg/g. The anthocyanin content decreased to 1,158 mg/g, and *T. koningii* increased the chlorophyll a and b content, total chlorophyll and carotenoids in the presence of *M. phaseolina* 22.471, 8.888, 7.151 and 6.927 mg/g and reduced the anthocyanin content to 1.878 mg/g. The bioagent fungi recorded the least significant effect against *R. solani*, the content of chlorophyll a, b, total chlorophyll, carotenoids were 14.172, 6.861, 6.556 and 6.986 mg/g, and anthocyanin content was reduced to 2.625 mg/g. Treatment of *C. globosum* increased chlorophyll a, b, total chlorophyll and carotenoids to 13.64, 6.391, 5.822 and 5.867 mg/g, respectively, and anthocyanin content decreased to 2.266 mg/g as compared to the treatment of pathogenic fungi alone (*T. koningii*). The results showed an increase in the stability index of chlorophyll due to the use of biological fungi, as it increased to 53.762% in the treatment of the interaction of *C. globosum* and *M. phaseolina*. The treatment of the fungus *C. globosum* and *F. solani* came

second, which reached 46.759%. The results of the statistical analysis indicated the significant effect of biological fungi in reducing the effect of the interaction between the concentrations of lead and cadmium and pathogenic fungi, as no significant differences were recorded for the interaction between the low concentrations of lead and cadmium and pathogenic fungi, while no significant effect of bioagent fungi was observed in reducing the effect of pathogenic fungi overlapping and high concentrations of lead and cadmium in comparison with the interaction treatments between pathogenic and bioagent fungi without lead and cadmium.

Trichoderma sp. can improve the production of plant pigments which may be attributed to its ability to colonize the roots of crop plants, this leads to the regulation of genes of pigments that improve photosynthesis of plants. Plants under physiological or environmental pressures suffer losses in their ability to photosynthesize. Inflicted by photosynthetic systems and other cellular processes produced by reactive oxygen species (ROS), but *Trichoderma* sp. activates biochemical pathways that reduce reactive oxygen species to less harmful molecules and other mechanisms described here make plants more resistant to biotic and abiotic stresses (Harman et al. 2019).

The fungus *Trichoderma* sp. stimulated the expression of two enzymes important to maintaining electron stress conditions and the NADH-dependent hydroperoxidase reductase, another enzyme involved in transporting a single carbon core unit to different biosynthesis pathways (Segarra et al. 2007). Doni et al. (2019) indicated that *Trichoderma* sp. stimulates many genes in the plant, including 238 genes associated with thylakoid membranes in chloroplasts and 192 genes related to photosynthesis. When attacking plant pathogens, it stimulates the formation of highly damaging reactive oxygen species ROS. These include the anion O₂, the hydroxyl radical OH, and hydrogen peroxide. They interact with proteins, fats and other plant metabolites to form free radicals that are highly damaging to cells and damage caused by free radicals to various cellular and random components, including denaturing proteins and DNA mutation and their effects are particularly harmful to cellular membranes (Nath et al. 2013). *T. harzianum* was able to control disease in tomatoes caused by *R. solani*, in part due to the stimulation of enzymes that inhibit (ROS) (Youssef et al. 2016). Inoculation of wheat with the fungus *T. harzianum* led to significant increases in water-soluble compounds, such as soluble sugars, soluble proteins, and proline (Zhang et al. 2019). The use of bioagent fungi *T. koningii* and *C. globosum* increased the ability of wheat plants to withstand the biological stresses of pathogenic fungi as well as the abiotic stress of lead and cadmium of fungi, which reflected positively on various traits such as seed germination percentage, seedling death, height and freshness and vegetative weight of the shoot and root system, as well as total phenols content in leaves, plant pigments, and chlorophyll stability index.

To summary, our results reassured the severity of wheat seed decay and seedling- damping-off caused by the fungal pathogens *R. solani*; *F. solani* and *M. phaseolina*; more severity indices were observed with the interaction between above- mentioned pathogens and heavy metals treatments with lead and cadmium. All examined parameters (morphological and biochemical features) were decreased significantly compared to control (untreated) ones. Applying bioagent fungi (*T. koningii* and *C. globosum*) leads to a significant decrease of each pathogenic and heavy metal on treated wheat plants. It is noteworthy that the bioagent fungus stimulated all examined parameters in treated wheat plants including the percentage of seed germination increased, damping-off decreased, as well as the increase in plant height. It was recorded that there was an increase in fresh and dry weight of shoot and root system. The bioagent fungi had increased the total phenol content, chlorophyll a and b, total chlorophyll, carotenoids content in leaves and chlorophyll stability. The current study recommends applying both *T. koningii* and *C. globosum* in biological control of wheat seed decay and seedling damping-off disease and heavy metal bioremediation.

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