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## EFFCIENCY OF SOME BIOLOGICAL AND CHEMICAL TREATMENTS AGAINST WHEAT ROOT AND CROWN ROT DISEASE

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ABSTRACT: Fusarium culmorum (W.G. Smith) Sacc., Bipolaris sorokiniana (Sacc.) Shoemaker, Rhizoctonia oryzae Kühn and Fusarium spp. were isolated from wheat plants exhibiting typical root and crown rot symptoms from different districts at Sharkia Governorate, Egypt during 2015/2016 growing season. Pathogenicity test revealed that B. sorokiniana was the most virulent one causing preemergence damping-off followed by Fusarium culmorum. In addition, F. culmorum was the most virulent one responsible for post-emergence damping-off incidence. Rhizoctonia oryzae showed the highest percentage of root rot, Moreover, F. culmorum and B. sorokiniana showed the highest percentage of disease incidence. In the same trend, F. culmorum induce the highest percentage of disease severity. In vitro, Trichoderma sp. bio-agent and its culture filtrate were the most effective treatment that reduced mycelial growth of the tested fungi. In vivo, it decreased pre and postemergence damping off, root rot, disease incidence and disease severity compared with the control. In addition, the obtained results indicated a significant increase on healthy survival plants and significantly improved the plant growth parameters *i.e.* fresh and dry weights of shoots and roots, plant height, spike length and 1000 grain weight. In vitro, Score and Amistar-top fungicides were the most effective in inhibiting the mycelial growth of R. oryzae followed by Amistar-top on B. sorokinana and Score on F. culmorum. Score was the most effective treatment revealed the highest percentage of healthy survival plants followed by Amistar-top and gave the highest protection against root and crown rot disease as shown by disease incidence and severity percentages.

Key words: Wheat, root rot, crown rot, biological control, chemical control.

### **INTRODUCTION**

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown worldwide and in Egypt. *Fusarium graminearum* and *F. culmorum* were the causal agent of root rot, crown rot and stem base disease in wheat (**Winter et al., 2019**). *Fusarium* spp. caused the two major diseases, fusarium head blight (FHB) and fusarium crown rot (FCR) on wheat that reduce yield qualitative and quality damage in addition to, a major mycotoxin procedure such as deoxynivalenol, zearalenone and nivalenol (**Matny, 2015; Mahmoud, 2016**). **Paulitz and Schroeder (2016)** reported that *Rhizoctonia oryzae* caused wheat and barley root rot, reduced emergence of wheat, moreover, reduced

and number length of roots. **Bipolaris** sorokiniana is a pathogen of cereals including, seedling blight, root rot, black point on grains, foliar spot, blotch, leaf blight and head blight on barley and wheat. It considered as seed borne fungi transmitted with seed (Burlakoti et al., 2013; Raza et al., 2014; Somani et al., 2019). Genus Streptomyces had high inhibition effect on Fusarium spp., in vitro, as well in vivo. It reduce root rot and crown colonization by F. culmorum, F. pseudograminearum. Results reported by Winter et al. (2019) significantly reduced fusarium crown rot symptoms on roots and fresh weight and plant biomass compared with enrichment soil by Streptomyces. Streptomyces spp. used as bio-fertilizers in several crops due to their ability to promote

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plant growth and biocontrol of various phytopathogenic fungi and bacteria as a reason of it is metabolizes, antibiotics and produce organic compounds in soil (Vurukonda et al., 2018). Clearly significant effect of actinomycetes were detected on F. culmorum, seedling growth and seed germination of wheat compared with commercial fungicide tebuconazole (Laid et al., 2016). Trichoderma harzianum, T. viens and T. virdi reduced mycelial growth of Bipolaris sorokiniana in dual culture method, as well as, significantly reduced disease severity, increased seed germination, plant height, dry fresh weight of root, shoot and increase 1000 grain weight (El-Gremi et al., 2017; Singh et al., 2018). Trichoderma spp. as a seed treatment reduced F. graminearum and F. culmorum root rot severity based on antagonistic activity against mycelium by mechanisms of mycoparasitism, antibiosis and chitinase encoding gene (Matarese et al., 2012; Xue et al., 2017). Trichoderma harzianum inhibited R. solani mycelium in vitro through dual culture technique (Rajendraprasad et al., 2017). Inoculate wheat plant with Trichoderma sp. as a seed and root treatments, reduced infection with Rhizoctonia sp., increased plant height and number of roots (Barnett et al., 2017). Seed coating treatment by plant growth-promoting rhizobacteria (PGPR) like Bacillus sp. and Pseudomonas sp., controlled wheat root and crown rot and increased significantly root length, root fresh weight, dry weight and shoot length (Moussa et al., 2013). Zhao et al. (2014) found that Bacillus subtilis isolated from wheat grains have antagonistic activity against F. graminearum mycelial growth, sporulation and toxin production as reason of destroying cell structure organelles and cytoplasm followed by cell death due to antifungal activity associated with production of chitinase and surfactins. Balah et al. (2018) reported that antifungal of some rhizobacterial activity isolates metabolites of Bacillus cereus and Pseudomonas geniculata were the most efficient isolates could be used as a good element to control Bipolaris sorokiniana in plant root rot disease. Bacillus sp. and Pseudomonas sp. were effective as biocontrol agent in vitro against F. culmorum, controlling fusarium head blight and reduced mycotoxin contamination on wheat (Dal Bello et al., 2002; Palazzini et al., 2016; Dweba et al., 2017; Mnasri et al., 2017). Pseudomonas spp. isolated from soil were active against wheat

root rot disease caused by *R. solani* and *R. oryzae*, it increased seedling root and shoot length (Mavrodi *et al.*, 2012).

Triazoles group such as Teubeconazole and Prothioconazole was the most effective fungicide for management Fusarium spp., F. culmorum, F. graminearum, F. cerealis and fusarium head blight of wheat. These group influence on ergosterol biosynthesis and considered most effective fungicide (Hellin et al., 2017; Shah et al., 2018). Also, Azole group of fungicides tebuconazole, prothioconazole, propiconazole and strobin (azoxystrobin) fungicides treatment showed increasing grain germination, plant height and decreased disease severity (%) caused by F. culmorum and Cochlibolus sativus in addition, inhibiting their mycotoxin production (Sooväli et al., 2017; Koycu, 2019). Score fungicides (Difenoconazole) was the best performance to inhibit Drechslera sorokiniana mycelial growth in poisoned food technique and seed treatment followed by Amistar-top (Azoxystropin + Difenoconazole) as mentioned by Mehboob et al. (2015).

Propiconazole as one of azole fungicides group used widely, its targets were the demethylase enzymes involved and inhibiting the biosynthesis of sterols which building blocks of fungal cell membranes. Propiconazole at 0.1 and 0.05% after 7 days was the most effective one for inhibition mycelial growth of Cochliobolus sativus and B. sorokiniana (Kavita et al., 2017; Somani et al., 2019). Tebuconazole and Difenoconazole as active ingredient were the most effective fungicide as seed treatment with a dose of 2.5 g and 1.0 ml/kg wheat grains, respectively and significantly increased the seedling emergence caused by B. sorokiniana and Fusarium sp., as compared with control. Moreover, reduced the number of rotted roots and healthy grains per spike and yield (Shahbaz et al., 2018). Propiconazole inhibited mycelium growth of R. solani (Rajput et al., 2016). Also, difenoconazole and azoxystrobin as new combination fungicide was effective against R. solani and R. oryzae (Bhuvaneswari and Raju, 2012; Kucharska et al., 2018).

Thus, this work was designed to isolate the causal pathogens of wheat root and crown rot. In addition, pathogenicity test, biological and chemical control treatment of the isolated pathogens and its effect on plant growth parameters.

### MATERIALS AND METHODS

### **Samples Collection**

Naturally infected wheat plants with root samples exhibit typical symptoms doubted to be due to root rot disease were collected from different districts at Sharkia Governorate (Zagazig, Kafr El-Hamam, Ghazala and Abu-Kaber). Samples were transferred under cooling using ice box to Plant Pathology Lab. Plant Pathology Dept., Fac. Agric., Zagazig Univ., Egypt.

## Isolation and Purification of the Pathogenic Fungi

The infected wheat roots were surface sterilized in 1% sodium hypochlorite solution for 2 minutes, rinsed twice in sterilized distilled water, dried between two sterilized filter papers. Then were cut into small pieces and transferred into water agar (WA) medium (**Parsons and Munkvold, 2012**) and incubated at 27±1°C for 7 days.

The developed fungi were recorded as frequency percentage for all the isolates and purified using the hyphal tip and/or single spore techniques (Skidmore and Dickinson, 1976; Dhingra and Sinclair, 1995). The purified fungi were transferred to potato dextrose agar (PDA) medium and kept slant at 5°C for identification and further studies.

### **Detection of the Isolated Fungi**

The isolated fungi from infected wheat samples were microscopically identified according to the morphological features of mycelia and asexual spores using the description of **Nelson** *et al.* (1983), Leslie and Summerell (2006) for *Fusarium* sp. and Manamgoda *et al.* (2014) for *Bipolaris sorokiniana*. The selected three isolates were identified at Plant Pathology Lab. Plant Pathology Dept., Fac. Agric., Zagazig Univ., Egypt.

### **Pathogenicity Tests**

Pathogenicity tests of four identified isolates of Fusarium culmorum, Fusarium spp., Rhizoctonia oryzae and Bipolaris sorokiniana were carried out under greenhouse conditions at Fac. Agric., Zagazig Univ.

### **Inoculum preparation**

Inoculum of *Fusarium* spp., *F. culmorum*, *R. oryzae* and *B. sorokiniana* prepared using autoclaved wheat grains (200 g of wheat grains, 80 ml distilled water per flask 500 ml) singly inoculated by each pathogen and incubated at  $27\pm1^{\circ}$ C for three weeks (**Chekali** *et al.*, 2011).

#### Pots and soil disinfestation

Sterilized plastic pots (25 cm in diameter) with formalin 3% for 10 min. were filled with 6.6 kg sterilized autoclaved sandy clay soil (1:1).

#### Soil infestation

Soil infestation was carried out by adding the fungal inoculum (5 g/kg soil) to the sterilized autoclaved soil. The infested soil was watered as usual and left for 10-15 days before sowing to stimulate the fungal growth and ensure its distribution in the soil. Control pots were treated in the same way using pathogen free autoclaved wheat grains described by El-Sayed (1999). Wheat grains Masr 1 cultivar were obtained from Filed Crop Research Institute, Agric. Res., Cent. (ARC). Grains were sterilized with 1% sodium hypochlorite solution for 2 minutes and sown at the rate of 10 grains /pot. Three replicates were used for each treatment. Inoculated fungi were re-isolated from the infected plants to confirm Kock's postulate.

#### **Disease Assessment**

Damping-off incidence was recorded as percentage of pre, post-emergence healthy survivals percentage at 15, 30 and 45 days after sowing, respectively. Crown and root rots severity was done on infected plants damping off mature plants. Infected plants were removed from pots, then washed and disease severity was rated on a 0 to 3 scale based on symptoms observed on the crown and roots, 0: no symptoms (healthy roots and crown); 1: browning on the crown; 2: extension of browning to roots and 3: dark brown color of the crown and all roots. Disease severity was averaged among the replicates according to Chekali et al. (2011). Disease severity was calculated using the scale values, as follows:

( $\sum$  (number of plants in a disease scale category  $\times$  disease scale category)/(total number of plants  $\times$  maximum disease scale category))  $\times$  100). The most virulent isolate was selected on the basis of disease severity averages.

### **Plant Growth Parameters**

Growth parameters including plant height (cm), fresh and dry weights (gram) were estimated at the end of experiment. Root system was tapped out of the pot and washed with gentle stream of water, for obtaining fresh and dry weight, the roots were pressed gently between two pads of blotting paper then the fresh weight was recorded using electronic balance. Dry weight was recorded after drying the roots and shoots in oven under 70°C for several days until the constant weight. Spike length (cm), spike number and weight of the 1000 kernels (gram) were determined.

### **Biological Control**

Plant growth promoting rhizobacteria (PGPR) isolates was obtained through isolation of biocontrol microorganisms from wheat rhizosphere by the serial dilution agar plating method using different selective media according to **Jacobs and Gerstein (1960).** Identification of isolated Fungi, Streptomyces and bacteria was carried out at Plant Pathology Lab. Plant Pathology Dept., Fac. Agric., Zagazig Univ., Egypt using identification roles mentioned by **Shirling and Gottlieb (1966); Lelliott and Stead (1987) and Krishna** *et al.* **(2012).** 

### **Laboratory Experiments**

### Evaluation the inhibitory effect of isolated bacteria on pathogenic fungi using dual culture technique

Antagonistic activities of identified Streptomyces sp., Bacillus sp., Pseudomonas sp. and Trichoderma sp. were done against wheat root rot pathogens were cultured for 7 days onto PDA medium, then 5 mm disc of the pathogenic fungi were re-cultured onto one side of 9 cm Petri dish and the opposite side was cultured with one disc Trichoderma sp. bioagent and/ or streak in the case of bacteria and Streptomyces sp. at the same. Three plates were used as replicates for each treatment. Mycelial discs (5 mm) of diameter removed from the growing edge of R. oryzae, F. culmorum and B.

sorokiniana grown onto PDA were used as control. The plates were incubated at  $28 \pm 2^{\circ}$ C for 5 days in the dark. Linear growth of pathogenic fungi was measured, when the control dishes reached full growth and the growth diameter average, was calculated. The inhibition rate of tested bio-agent on mycelial growth pathogens was calculated using the following formula: growth inhibition rate (%) = (Rc-Rt)/RC×100, where Rc is the average linear growth of pathogen (control), and Rt is the average growth of the pathogen. Four Petri dishes for each antagonist were used (Skidmore and Dickinson, 1976).

### Influence of antagonist bio agents culture filtrates on wheat root rot pathogen

Antagonist cultures were grown in flasks (250 ml) containing 100 ml potato dextrose broth (PDB) for one week at 25°C., nutrient agar (NA) used for bacteria and starch nitrate (SN) media for actinomycetes. Pseudomonas sp. and Bacillus sp. increased the turbidity (silkiness or cloudiness). The liquid cultures were filter sterilized using G3 filter to give sterile and cell free culture filtrate according to Jacobs and Gerstein (1960). Culture filtrates were mixed with autoclaved PDA media before solidifying (at 1%) and poured in Petri plates. Then plats were inoculated with a disc (0.5 cm diameter) of the pathogens and incubated at 25°C for one week. Inhibition of mycelial growth of F. culmorum, R. oryzae and B. sorokiniana were calculated.

### Biological control using isolated plant growth promoting microorganism's against wheat root rot and growth parameter under greenhouse conditions

In planta, healthy wheat grains cv. Masr 1 were surface sterilized in 1% NaOCl (Sodium hypochlorite) for 2 minutes and then rinsed three times in sterile water to get rid of surface seed secondary fungal pathogens as mentioned by **Parsons and Munkvold (2012)**. Then wheat grains cv. Masr-1 were soaked in 50 ml suspension of *Trichoderma* sp. at  $1.50 \times 10^{-6}$  spores/ml for 2 hr. Wherever, in case of bacterial bioagent (*Pseudomonas* sp., *Bacillus* sp. and *Streptomyces* sp.) wheat grains were dipped in 50 ml suspension at  $6 \times 10^{-8}$  cfu/ml for 2 hr. (Ma *et al.*, 2008).

For control treatments, Grains were soaked individually with sterile water for 2 hr, in 50 ml according to **Xue** *et al.* (2017). Treated grains were air dried and ten grains were planted in pots (25 cm) containing infested and noninfested soils as control. Three pots were used for each particular treatment. Seed soaking in sterile distilled water were sown in infested and non-infested soil to serve as positive and negative control, respectively. Disease parameter and plant growth parameter were recorded as previously mentioned.

### **Chemical Control**

## Evaluation the inhibitory effect of some fungicides on the linear growth of pathogenic isolates *in vitro*

Three tested fungicides Score 250 EC (Difenoconazole 25%), Amistar-top 325 SC (Azoxystrobin 20% and Difenoconazole 12.5%) and Topas 100 EC (Penconazole 10%) as shown in Table 1 were obtained from Plant Pathology Lab. Plant Pathology Dept., Fac. Agric. Zagazig Univ., Egypt. Fungicides were used at different concentrations (0.00, 0.025, 0.05, 0.10, 0.15 and 0.20 ml) against F. culmorum, R. oryzae and B. sorokiniana using poison food technique according to Kavita et al. (2017) on potato dextrose agar (PDA) medium. Linear growth of each tested fungus was measured, when the pathogenic fungi completely covered the surface of the medium in control treatment. The radial growth of fungus in each treatment (percent growth inhibition) was calculated using the following formula. [PGI= C-T/C]×100, Where, PGI = Percent growth inhibition; C = Lineararea of test fungus in control (mm) and T =Linear area of test fungus in respective treatment (mm) according to Rajendraprasad et al. (2017).

## Evaluation of some fungicides against *F*. *culmorum*, *R*. *oryzae* and *B*. *sorokiniana* as seed treatment under greenhouse conditions

Fungicides were applied at the recommended dose (0.025 ml/kg grains). Grains of wheat was surface sterilized as mentioned before then separately mixed with the recommended dose of each fungicide. Treated grains were left to dry. Ten grains of Masr 1 cultivar planted in pots (25 cm diameter) previously infested with the pathogenic fungi as previously mentioned and irrigated after planting. The recommended rate of fertilizers was applied. The experiment was conjunctly randomized block design (RBD) with three replicates.

### **Statistical Analysis**

All experiments were conducted in a factorial (treatments× three fungal species) a completely randomized block design with three replicates per treatment. Analyzed was carried out according to the methods described by **Snedecor and Cochran (1980)** using Statistic Complete 9 Program for ANOVA and LSD analysis.

### **RESULTS AND DISCUSSION**

### **Samples Collection**

The samples exhibit typical disease symptoms of wheat root and crown rot were collected. The disease reduced weight of grains, Infection increased in high or semi high humidity (Shah *et al.*, 2018; Winter *et al.*, 2019).

Also, some of pathogenic fungi such as, *Rhizoctonia* spp., *R. oryzae* and *R. oryzae* AG-8 consider a necrotrophic pathogen that infect wheat and barley symptoms was chronic root rot, causing pre-emergence damping off, crown roots in seedlings, stunted plants, reduced plant growth length, reduced length and number of seminal roots and bare patch decreased losses in growth and yield as well as, number of grains on spike (**Paulitz and Schroeder, 2016**).

### Isolation, Identification and Frequency of Fungi Associated with Infected Wheat Grains

Identification the isolated fungi based on morphological characterization was used as it has been previously utilized in various other studies for *Fusarium* sp. and *Bipolaris sorokiniana* (Nelson *et al.*, 1983; Leslie and Summerell, 2006; Manamgoda *et al.* 2014).

The obtained results, diagnosed fungal isolates associated with wheat root rot, as *Fusarium culmorum* (W.G. Smith) Sacc., *Bipolaris sorokiniana* (Sacc.) Shoemaker, *Rhizoctonia oryzae* Kühn and *Fusarium equestii*.

El-Enany, <i>et al</i> .	
Table 1. List of the tested fungicides, their active ingredients, manufacture and rate of use	

Commercial fungicide	Active ingredient	Manufacture	Rate of used (cm/100 L)
Score 250 EC	Difenoconazole 25%	Syngenta	50
Amistar-top 325 SC	Azoxystrobin 20%	Syngenta	75
	Difenoconazole 12.5%		
Topas 100 EC	Penconazole 10%	Syngenta	25

Results presented in Fig. 1 show that the most frequently isolated fungi from infected wheat root and crown samples were *R. oryzae*, *F. culmorum* and *B. sorokiniana* (27.80, 27.40 and 21.56%, respectively).*R. oryzae* was the most frequently isolate one especially from Kafr El- Hamam (53.33%) followed by, *F. culmorum* in Zagazig (50.94%). and *B. sorokiniana* from Abu-Kabeer (31.15%).

Similar results were showed by **Tunali** *et al.* (2008) that frequency of the isolated fungi from crowns and roots of wheat in dryland was reported as *Rhizoctonia* species found in 22%, *F. culmorum* (14%), *B. sorokiniana* (10%) and *F. pseudograminearum* (2%). In addition, they isolated fungi from individual tillers which were *B. sorokiniana* (15%), *F. culmorum* (13%) and *F. pseudograminearum* (8%).

Also, **Abdallah-Nekache** *et al.* (2019) reported that frequency of isolated fungi from wheat crown was 68% to *F. culmorum* and 10% for *F. pseudograminearum*. While, isolation from head was 94.1% to *F. culmorum* and 5.9% to *F. pseudograminearum*.

### **Pathogenicity Tests**

Results obtained from pathogenicity tests under greenhouse conditions in Fig. 2 reveal that significant differences were found between tested fungi. The highest percentage of preemergence damping-off was recorded with *B. sorokiniana* followed by *F. culmorum* and *R. oryzae*. Also, *Fusarium culmorum* produced the highest percentage of post-emergence dampingoff. *R. oryzae* showed the highest percentage of root rot followed by *B. sorokiniana* and *F. culmorum* without significant differences among them. In addition, the highest percentage of disease incidence were found in both *F. culmorum*, *B. sorokiniana* and *R. oryzae*. While, *F. equestii* was the lowest one. Disease severity was in the highest level in case of *F. culmorum* followed by *B. sorokiniana* and *R. oryzae*. As well, *Fusarium equestii* showed the highest percentage for survival healthy plants.

Similar results were obtained by Gebremariam *et al.* (2018) and Abdallah-Nekache *et al.* (2019) where they reported that *Fusarium culmorum* was the most aggressive pathogen on wheat from seedling to heading. In addition, *Fusarium* species infected lower stems and crown. *F. culmorum*, *F. graminearum* and *F. pseudogramineaum* caused sever crown rot on wheat. They found also that, *Cochilibolus sativus*, *F. graminearum*, *F. culmorum* and *F. avenaceum* were virulent to wheat and barley.

Fusarium sp. produced micro-conidia in colossal amounts, which are known to transmit through air to large distances, finally infecting wheat roots and crown parts of plant as mentioned by Leslie and Summerell, (2006). B. sorokiniana as a hemi-biotroph pathogen it caused symptoms *i.e.* seedling blight, brown to dark brown spot, foliar blotch, leaf blight, root rot and black point of wheat. It considered transmitted with seed as seed borne fungi. In addition, it affecting significantly on seed germination and head blight on barley and wheat, as well, Bipolaris sorokiniana caused rot at the sub crown internode (Al-Sadi and Deadman, 2010; Raza et al., 2014; Somani et al., 2019).

### **Biological Control**

### *In vitro* effect of some bioagent using dual culture technique against *Fusarium culmorum*, *Bipolaris sorokiniana* and *Rhizoctonia oryzae*

Results presented in Figs. 3 and 4 indicate that, the bio-agents tested significantly reduced growth of the tested pathogenic fungi *i.e.* 





Fig. 1. Frequency of occurring isolated fungi from root and crown rot of wheat collected from different districts of El Sharkia Governorate during 2015/2016 growing seasons



Fig. 2. Pathogenicity test for the isolated fungi that caused wheat root and crown rot disease of Masr 1 cultivar under greenhouse condition



A- Trichoderma sp. against Fusarium culomrum



B- Streptomyces sp. against Bipolaris sorokinana



C-Bacillus sp. against Rhizoctonia oryzae

Fig. 3. Biological control using dual culture technique



## Fig. 4. Reduction percentage in the mycelial growth of the causal organisms of wheat root and crown rot disease using dual culture technique of the tested bio-agents

B. sorokiniana followed by F. culmorum and R. oryzae. Trichoderma sp. showed the highest reduction percentage of the mycelial growth of tested pathogenic fungi followed by Streptomyces isolate 1 and Streptomyces isolate 2. However, Bacillus sp. was the least effective one. In addition, Trichoderma sp. was the most effective on R. oryzae followed by Bacillus sp. Although, Streptomyces isolate 2 was less effective. As well, Trichoderma sp. showed the percent highest reduction followed by Streptomyces isolate 2. While, *Pseudomonas* sp. was the less effect one on F. culmorum. In case B. sorokiniana, Trichoderma sp. was the highest effective one followed by Streptomyces isolated 1. Also, Bacillus sp., was the lowest one.

### *In vitro* effect of antagonist culture filtrates against the wheat root rot pathogen

Results in Fig. 5 show that *B. sorokiniana* was the highest sensitive fungus to culture filtrate followed by *F. culmorum*, while *R. oryzae* was the lowest one (50.41, 41.78 and 33.72%, respectively). *Trichoderma* sp. culture filtrate was the most effective on reducing fungal growth followed by *Bacillus* sp. and *Streptomyces* isolate 1 (72.85, 63.63 and 43.37%, respectively).

# The effect of different bio-agent *Streptomyces* spp., *Pseudomonas* sp., *Bacillus* sp., and *Trichoderma* sp. on wheat root and crown rot disease and plant growth parameter under greenhouse conditions

Data in Table 2 indicate that results of both dual culture and culture filtrate were in

harmony. Trichoderma sp. showed the highest percentage of healthy survival plants followed by Streptomyces isolate 1. While Pseudomonas sp. performed the lowest compared to control. There were significant differences between bioagent on pre-emergence damping off whereas, Trichoderma sp. reduced pre-emergence, followed by Streptomyces isolate 2. While, Pseudomonas sp. showed the highest percentage of preemergence, Trichoderma sp. and Streptomyces isolate 1 showed the lowest percentage of postemergence. Trichoderma sp. showed the lowest percentage of disease parameters (root rot, disease incidence and disease severity). Such results consequently followed by significant high values of plant growth parameter (Table 3). Trichoderma sp. showed the highest value of root fresh weight (3.92 g), shoot length (80.00 g) and 1000 kernel weight (48.73 g), followed by Bacillus sp. for shoot fresh weight (19.94 g) and spike length (11.18 cm) compared to control. Treatment of plant growth promoting rhizobacteria (PGPR) increased seed germination and shoot/ root growth might be due to IAA, gibberellins and cytokinin production. Other mechanism such as siderophores, hydrocyanic acid and induction of resistance may play a role in the mode of action of PGPR (Singh et al., 2015). Thus, rhizobacterial agents considered to be one of the most significant strategies for disease management (Laid et al., 2016). Antagonistic activity of *Streptomyces* against plant pathogens attained through different mechanisms, i.e.

production of secondary enzymatic activities metabolites including nutrient competition, chitinase, antibiosis, induced resistance, production of degradative enzymes, and nitrous oxide production (Cohen and Mazzola, 2006; Mahmoudi *et al.*, 2011; Boukaya *et al.*, 2018; Winter *et al.*, 2019).

Streptomycetes was an active producer of volatile organic compounds and antibiotics both in soil and in planta, and this feature was helpful for antagonists of plant pathogens as biocontrol agents. Production of siderophores (iron-chelating compounds) and chitinolytic enzymes as mode of action for fungal growth inhibition by endophytic actinobacteria. In addition to produce enzymes that degrade fungal cell walls by the production of chitinases. Moreover, actinomycetes present 90% of chitinolytic microorganisms (**Vurukonda** *et al.*, **2018**).

The biological inhibition ability of selected Pseudomonas spp. might be as reason of competition for space and nutrients, siderophore mediated competition for iron, induction of induced systemic resistance and antibiosis in the host plant (Balah and Latif, 2013). Strains of some Bacillus sp. had the ability to produce chitinolytic enzymes and to induce systemic resistance in the host plant (Tsai et al., 2002; Moussa et al., 2013). Bacillus subtilis have antagonistic activity against F. graminearum mycelial growth, sporulation, toxin production and reduced disease incidence by production of chitinase and surfactants and broad spectrum of antimicrobial compounds. Similar result was mentioned by Cohen- Kupiec (1998) and Zhao et al. (2014).

Balah et al. (2018) found that the secondary metabolites in case of Pseudomonas geniculata coumaric acid, aminobutyric acid, were tryptophan amino acid, 1,4-benzoquinone, succinic acid, sinapic acid and ferulic acid. However, B. cereus produced 1,4-benzoquinone, aminobutyric acid, ferulic acid benzoic acid, coumaric acids and sinapic acid. Similar result was matched with Singh et al. (2018) who found that Trichoderma harzianum, T. virdi and T. viens on mycelial growth of B. sorokiniana which increased plant height, fresh and dry weights of shoots and roots of wheat seedlings compared with the control, Moreover, the hyphal interaction between test fungus and revealed disorganization Antagonists of protoplasmic content in addition, lysis of host hyphae. Trichoderma harzianum improved germination, seedling growth, length of roots, shoots, tillers and increase 1000 grain weight in wheat disease caused by C. sativus and F. graminearum. T. harzianum showed hyperparasitism on the tested pathogens *i.e.* F. graminearum, C. sativus and A. alternata in dual culture assays (El-Gremi et al., 2017). Trichoderma isolates reduced inoculum and growth of *F. culmorum* and *F. graminearum* by mechanisms of mycoparasitism, chitinase and encoding gene antibiosis. Furthermore, it coil around the pathogens hyphae, which is considered a sign of mycoparasitism (Matarese et al., 2012).

### **Chemical Control**

## Inhibitory effect of some fungicides of tested pathogenic fungus on linear growth

Results in Table 4 show that *R. oryzae* was the most sensitive to fungicide followed by *F. culmorum* and *B. sorokiniana*. Also, Results showed that Score highly reduced the mycelial growth of *F. culmorum* followed by Topaz while, Amistar-top was the less effective one. No significant differences were observed between tested concentrations (0.025, 0.05, 0.1, 0.15 and 0.2 ml) on *R. oryzae* with both Score and Amistar-top.

### Evaluation of fungicides against *F. culmorum*, *R. oryzae* and *B. sorokiniana* under greenhouse conditions

Results in Tables 5 and Fig. 6 indicat that Score and Amistar-top fungicides showed the lowest percentage of pre and post-emergence without significant differences between them followed by Topaz compared to control. Score showed the lowest percentage of root rot and highest percentage of healthy plants followed by Amistar-top and Topaz. There were significant differences among treatment fungicides Score, Amistar-top and Topaz in disease incidence (20.00, 22.50 and 24.53%, respectively). Score showed the lowest percentage of disease severity (8.33%) followed by Amistar-top (14.99%). While, the highest percentage was observed with Topaz (16.57%).



Fig.	5.	Reduction	percentage	in the	mycelial	growth	of the	causal	organisms	of	wheat	root	and
		crown rot	disease due	to cultu	ire filtrat	tes of the	e tested	l bio-ag	gents				

Table 2. In vive	effect o	of some	bio-agent	treatments on	wheat	root and	crown	rot p	pathogens	on
pathog	genic pai	rameters	s wheat Ma	asr1 cultivar						

Treatment		]	Pathoge	enic paramo	eters	
	<b>Pre</b> (%)	Post	Root	Disease	Disease	Healthy
		(%)	rot	incidence	severity	(%)
			(%)	(%)	(%)	
Fusarium culmorum	26.67	30.00	43.33	90.00	85.20	0.00
Rhizoctonia oryzae	23.33	10.00	60.00	70.00	68.40	6.67
Bipolaris sorokinana	30.00	10.00	60.00	90.00	73.33	0.00
Control	0.00	0.00	0.00	0.00	0.00	100.0
Average	20.00	12.50	40.83	62.50	56.73	26.67
Streptomyces isolate 1	0.00	0.00	0.00	0.00	0.00	100.0
Streptomyces isolate 1+Fusarium culmorum	16.67	0.00	33.33	55.00	20.56	50.00
Streptomyces isolate 1+Rhizoctonia oryzae	6.67	0.00	26.66	50.00	34.89	66.67
Streptomyces isolate 1+Bipolaris sorokinana	6.67	0.00	16.60	30.00	18.05	76.67
Average	7.50	0.00	19.16	33.75	18.38	73.34
Streptomyces isolate 2	0.00	0.00	0.00	0.00	0.00	100.0
Streptomyces isolate 2+Fusarium culmorum	20.00	3.33	36.67	40.00	23.33	40.00
Streptomyces isolate 2+Rhizoctonia oryzae	3.33	0.00	36.67	40.00	23.33	60.00
Streptomyces isolate 2+Bipolaris sorokinana	3.33	3.33	26.67	40.00	33.33	66.67
Average	6.67	1.67	25.00	30.00	19.99	66.67
Pseudomonas sp.	0.00	0.00	0.00	0.00	0.00	100.0
Pseudomonas sp.+Fusarium culmorum	23.33	3.33	33.33	60.00	23.33	40.00
Pseudomonas sp.+Rhizoctonia oryzae	13.33	0.00	13.33	20.00	16.66	73.33
Pseudomonas sp.+Bipolaris sorokinana	6.67	0.00	43.33	60.00	40.00	50.00
Average	10.83	0.83	22.50	35.00	19.99	65.83
Bacillus sp.	0.00	0.00	0.00	0.00	0.00	100.0
Bacillus sp.+Fusarium culmorum	16.67	0.00	30.00	30.00	24.33	53.33
Bacillus sp.+Rhizoctonia oryzae	6.67	0.00	23.33	30.00	20.33	70.00
Bacillus sp.+Bipolaris sorokinana	13.33	3.33	33.33	40.00	30.00	50.00
Average	9.17	0.83	21.67	25.00	18.67	68.33
Trichoderma sp.	0.00	0.00	0.00	0.00	0.00	100.0
Trichoderma sp.+Fusarium culmorum	13.33	0.00	13.33	20.00	10.50	73.33
Trichoderma sp.+Rhizoctonia oryzae	3.33	0.00	23.33	50.00	33.33	73.33
Trichoderma sp.+Bipolaris sorokinana	6.67	0.00	16.66	18.50	16.66	76.67
Average	5.83	0.00	13.33	22.13	15.12	80.83
LSD 0.05%						
A- Fungi	0.66	0.26	0.29			0.72
<b>B-Bioagent</b>	0.54	0.18	0.47			0.85
C- interaction	1.09	0.37	0.95			1.70

Treatment	Plant growth parameter												
	Root dry	Shoot fresh	Shoot dry S	Shoot lengt	h Root length	Tiller	Spike	Kernels	1000 kernel	Head			
	weight (g)	weight (g)	weight (g)	(cm)	(cm)	(No.)	length (cm)	number (No.)	weight (g)	blight (No.)			
Fusarium culmorum	1.15	12.50	1.24	68.00	12.00	2.00	8.90	39.67	25.70	2.00			
Rhizoctonia oryzae	1.65	15.27	4.13	73.00	14.00	2.00	9.40	35.33	37.80	0.00			
Bipolaris sorokinana	1.18	13.22	2.29	70.00	14.00	2.00	10.10	42.33	39.50	0.00			
Average	1.46	14.91	3.74	72.25	14.00	2.25	9.75	41.00	36.48	0.50			
Streptomyces isolate 1	2.43	18.30	7.03	79.00	18.00	3.00	10.87	59.67	48.10	0.00			
Streptomyces isolate 1+Fusarium culmorum	2.07	17.18	5.95	76.00	16.00	3.00	10.50	49.00	45.70	0.00			
Streptomyces isolate 1+Rhizoctonia oryzae	1.76	16.03	5.10	76.00	16.00	3.00	10.33	47.00	44.20	0.00			
Streptomyces isolate 1+Bipolaris sorokinana	2.19	17.95	5.68	77.00	17.00	3.00	10.80	56.76	47.80	0.00			
Average	2.11	17.37	6.19	77.00	16.75	3.00	10.63	53.17	46.45	0.00			
Streptomyces isolate 2	2.68	19.21	7.68	79.00	18.00	3.00	10.87	56.33	47.80	0.00			
Streptomyces isolate 2+Fusarium culmorum	2.54	17.63	6.74	//.00	16.00	3.00	10.67	50.67	45.80	0.00			
Streptomyces isolate 2+Rhizoctonia oryzae	1.61	15.57	5.11	76.00	15.00	3.00	10.27	44.67	41.10	0.00			
Streptomyces isolate 2+Bipolaris sorokinana	2.62	18.00	6.94	78.00	17.00	3.00	10.67	53.33	46.60	0.00			
Average	2.36	17.60	6.62	77.50	16.50	3.00	10.62	51.25	45.33	0.00			
Pseudomonas sp.	2.68	17.54	6.97	79.00	18.00	3.00	11.27	60.00	48.70	0.00			
Pseudomonas sp.+Fusarium culmorum	2.51	17.16	6.50	78.00	17.00	2.00	11.23	54.67	47.20	0.00			
Pseudomonas sp.+Rhizoctonia oryzae	2.57	17.29	6.64	78.00	17.00	3.00	11.10	57.33	47.90	0.00			
Pseudomonas sp.+Bipolaris sorokinana	2.49	16.91	6.43	78.00	16.00	2.00	10.50	55.00	46.40	0.00			
Average	2.56	17.23	6.64	78.25	17.00	2.50	11.03	56.75	47.55	0.00			
Bacillus sp.	3.21	20.74	9.65	80.00	18.00	2.00	11.33	57.33	50.10	0.00			
Bacillus sp.+Fusarium culmorum	2.30	20.33	6.34	79.00	17.00	2.00	11.17	54.67	49.60	0.00			
Bacillus sp.+Rhizoctonia oryzae	2.35	19.45	8.55	78.00	16.00	2.00	11.23	52.33	47.70	0.00			
Bacillus sp.+Bipolaris sorokinana	2.41	19.24	8.32	78.00	17.00	2.00	10.97	49.33	46.20	0.00			
Average	2.57	19.94	8.97	78.75	17.00	2.00	11.18	53.42	48.40	0.00			
Trichoderma sp.	3.03	20.83	9.53	81.00	18.00	3.00	11.33	58.33	51.40	0.00			
Trichoderma sp.+Fusarium culmorum	2.95	20.17	8.90	80.00	18.00	3.00	11.27	52.33	49.50	0.00			
Trichoderma sp.+Rhizoctonia oryzae	1.91	17.06	6.89	79.00	17.00	3.00	10.90	45.67	46.80	0.00			
Trichoderma sp.+Bipolaris sorokinana	2.69	18.74	7.54	80.00	18.00	3.00	11.17	48.67	47.20	0.00			
Average	2.65	19.20	8.22	80.00	17.75	3.00	11.17	51.25	48.73	0.00			
Un treated	1.87	18.63	7.29	78.00	16.00	3.00	10.60	46.67	42.90	0.00			
LSD 0.05%		'											
A- Fungi	0.38	0.60	0.49	0.49	0.55		0.31	3.12					
B-Bioagent	0.39	0.48	0.43	0.69	0.66		0.30	3.73					
C- interaction	0.78	0.96	0.87	1.38	1.32		0.60	7.47					

Table 3. In vivo effect of some bio-agent treatments on wheat root and crown rot pathogens on plant growth parameters of wheat cultivar Masr1

Concentration (ml)		Rhizoctoni	a oryzae			Fusarium ci	ulmorum	l		Bipolaris sor	okiniand	ı
	Score	Amistar top	Topaz	Average	Score	Amistar top	Topaz	Average	Score	Amistar top	Topaz	Average
0.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.025	94.44	94.44	70.78	86.55	72.22	62.22	66.00	66.81	70.78	88.56	71.89	77.08
0.05	94.44	94.44	94.44	94.44	77.78	72.22	82.78	77.59	74.11	94.44	94.44	87.66
0.1	94.44	94.44	94.44	94.44	88.33	75.56	84.78	82.89	87.44	94.44	94.44	92.11
0.15	94.44	94.44	94.44	94.44	88.89	79.44	87.00	85.11	92.78	94.44	94.44	93.89
0.2	94.44	94.44	94.44	94.44	94.44	85.22	88.56	89.41	93.89	94.44	94.44	94.26
Average	78.70	78.70	74.76		70.28	62.44	68.19		69.83	77.72	74.94	

Table 4. In vitro inhibitory evaluation of some fungicides on reduction percent of the treated pathogenic fungi

LSD 0.05% A- Concentration 0.15 B-Fungi 0.10 C-Fungicides 0.10

Treatment	nt Pre emergence damping off (%)				ff (%)	Post emergence damping off (%)				Root rot (%)				Healthy survival plants (%)						
	Fusarium culmorum	Rhizoctonia oryzae	Bipolaris sorokiniana	Control	Average	Fusarium culmorum	Rhizoctonia oryzae	Bipolaris sorokiniana	control	Average	Fusarium culmorum	Rhizoctonia oryzae	Bipolaris sorokiniana	control	Average	Fusarium culmorum	Rhizoctonia oryzae	Bipolaris sorokiniana	control	Average
Without	26.67	23.33	30.00	0.00	20.00	30.00	10.00	10.00	0.00	12.50	43.33	60.00	60.00	0.00	40.83	0.00	6.67	0.00	100.0	26.67
Score	3.33	00.00	6.67	00.00	2.50	00.00	00.00	00.00	00.00	00.00	16.66	6.67	6.66	0.00	7.50	80.00	93.33	86.67	100.0	90.00
Amistar- top	00.00	3.33	6.67	00.00	2.50	00.00	00.00	00.00	00.00	00.00	23.33	10.00	10.00	0.00	10.83	76.67	86.67	83.33	100.0	86.67
Topaz	3.33	3.33	6.67	0.00	3.33	3.33	00.00	3.33	0.00	1.67	33.33	33.33	26.67	0.00	20.83	60.00	73.33	63.33	100.0	74.17
Average	8.33	7.50	12.50	0.00		8.33	2.50	3.33	0.00		29.16	25.00	25.83	0.00		54.17	65.00	58.33	100.0	
L.S.D. 0.05%																				
A- Fungi		0.14					0.36					0.39					0.39			
<b>B-Fungicide</b>		0.41					0.43				•	0.63					0.67			
<b>C-Interaction</b>		0.83					0.87					1.26					1.35			

Table 5. Effect of fung	gicidal treatments on	disease parameters of	of wheat root rot pat	thogens under g	reenhouse condition

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Fig. 6. Effect of fungicidal treatments on disease incidence and severity percentage of wheat root rot pathogens under greenhouse condition

Table 6. Evaluation of fungicides against F. culmorum, R. oryzae and B. sorokiniana undergreenhouse conditions on plant growth parameters on Masr 1 cultivar

Treatment Plant growth parameter												
Fungicide	-	Root dry weight(g)	Shoot fresh weight(g)	Shoot dry weight(g)	Shoot Length(cm)	Root length (cm)	Tiller (No)	Spike length (cm)	Kernels number (No)	1000 kernels weight (g)	Head blight (No)	
	Control	2.83	19.91	8.88	81.00	19.00	3.00	11.00	61.33	47.70	0.00	
re	Fusarium culmorum	2.58	19.10	8.08	79.00	17.00	3.00	10.90	51.33	46.20	0.00	
00)	Rhizoctonia oryzae	2.64	19.64	8.64	79.00	18.00	3.00	11.10	54.33	46.70	0.00	
	Bipolaris sorokinana	2.71	19.65	8.56	80.00	18.00	3.00	11.23	57.00	47.09	0.00	
	Average	2.69	19.58	8.54	79.75	18.00	3.00	11.08	55.10	46.92	0.00	
do		2.92	19.87	1.21	80.00	18.00	3.00	11.25	01.55	47.40	0.00	
mistar to	Fusarium culmorum	2.34	17.86	6.89	78.00	16.00	2.00	10.90	49.33	45.30	0.00	
	Rhizoctonia oryzae	2.65	19.16	8.47	78.00	17.00	3.00	11.03	56.33	46.50	0.00	
Am	Bipolaris sorokinana	2.72	19.38	8.56	79.00	17.00	3.00	11.10	55.67	46.90	0.00	
ł	Average	2.66	19.07	7.78	78.75	17.00	2.75	11.07	55.67	46.53	0.00	
	Control	2.79	19.73	8.13	80.50	19.00	3.00	11.02	61.10	47.61	0.00	
N	Fusarium culmorum	2.52	18.98	7.95	80.00	17.00	3.33	11.08	50.74	46.05	0.00	
opa	Rhizoctonia oryzae	2.54	19.12	8.52	79.00	18.00	3.67	11.12	56.20	46.21	0.00	
Ţ	Bipolaris sorokinana	2.65	19.41	8.47	80.00	18.00	4.00	11.19	55.59	47.01	0.00	
	Average	2.63	19.31	8.27	79.88	18.00	3.50	11.10	55.91	46.72	0.00	
	Control	2.84	19.79	8.14	80.58	18.00	3.00	11.14	61.24	47.52	0.00	
•	A- Fungi	0.37	0.48	0.47	0.82	0.70		0.39	4.68			
05%	B-Fungicide	0.30	0.79	0.49	0.66	0.57		0.40	3.84			
LSL	C-(AB) Interaction	0.60	1.59	0.98	1.33	1.14		0.80	7.69			

Such results consequently followed by significant high parameters of plant growth illustrated in Table 6 that, Score revealed the highest value for each of root fresh weight (3.93 g), shoot fresh weight (19.58 g), spike length (11.08 cm) and 1000 grain weight (46.92 g), control produced, the lowest value for plant growth parameters.

Fungicides with the active substances of Tebuconazole, Propiconazole, Difenoconazole and Strobin (Azoxystrobin) groups has systemic features and commonly effectively used in the fungicide applications to seeds and leaves. These fungicides inhibit biosynthesis of ergosterol which plays an essential role in the cell membrane of the fungi and inhibiting fungal development by causing excess electrolyte loss (Akgul and Erkilic, 2016; Koycu, 2019). Moreover, it increased the germination of seeds, plant height and decreased the severity of the disease in wheat plants. Azole group, inhibited mycelial growth and reduced mycotoxin of F. graminearum, F. culmorum by affecting one or more than one places within a fungal cell (Dekker, 1982; Paul et al., 2008). Somani et al. (2019) explained propiconazole mode of action was by its inhibiting the biosynthesis of sterols of Cochliobolus sativus which building blocks of fungal cell membranes and the demethylase enzymes involved.

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## كفاءة بعض معاملات المكافحة الحيوية والكيميائية ضد مرض عفن الجذور والتاج في القمح أحمد محمد العناني- إنتصار السيد عبد النبي عباس - محمد أمين عبدالمنعم زايد - محمود محمد عطيه

قسم أمراض النبات – كلية الزراعة – جامعة الزقازيق – مصر

عزلت فطريات فيوز ايوم كولمورم، بيبو لارس سوروكينينا، ريزوكتونيا أوريزا، فيوز اريوم، من نباتات القمح التى ظهر عليها أعراض الإصابة بعفن الجذور والتاج من مناطق مختلفة من محافظة الشرقية خلال الموسم الزراعي ٢٠١٥/ الظهور فوق سطح التربة، تلاه فطر فيوز ايوم كولمورم، بينما كان فطر فيوز اريوم كولمورم الأكثر ضراوة فى حالة موت البادرات ما بعد الظهور فوق سطح التربة، أظهر فطر ريز وكتونيا أوريزا النسبة الأعلى لعفن الجذور، في حين كان فطرى فيوز ايوم كولمورم و بيبو لارس سوروكينينا كان فطر فيوز اريوم كولمورم الأكثر ضراوة فى حالة موت البادرات ما بعد الظهور فوق سطح التربة، أظهر فطر ريز وكتونيا أوريزا النسبة الأعلى لعفن الجذور، في حين كان فطرى فيوز ايوم كولمورم و بيبو لارس سوروكينينا الأكثر في حالة نسبة الإصابة، بينما كان فطر فيوز ايوم كولمورم الأعلى فى فيوز ايوم كولمورم و بيبو لارس سوروكينينا وريزا منه مزرعته الأكثر تأثير أعلى تنثيط النمو الميسليومى للفطريات فيوز ايوم كولمورم و بيبو لارس سوروكينينا وريز وكتونيا أوريزا، كما أظهر فطر تر ايكودرما نتائج واضحة فى تقليل موت البادرات ما قبل الظهور فوق سطح التربة وموت البادرات بعد الظهور فوق سطح التربة و عفن الجذور ونسبة وشدة الإصابة، معمليا كان فطر تر ايكودرما وريز وكتونيا أوريزا، كما أظهر فطر تر ايكودرما نتائج واضحة فى تقليل موت البادرات ما قبل الظهور فوق سطح التربة وموت البادرات بعد الظهور فوق سطح التربة و عفن الجذور ونسبة وشدة البادرات ما قبل الظهور فوق سطح التربة وموت البادرات بعد الظهور فوق سطح التربة و عفن الجذور ونسبة وشدة السبادية، وقد كان لتلك المعاملات تأثير إيجابي ومعنوي على صفات نمو النبات والإنتاج (الوزن الجاف والرطب لكل من المليمة، وقد كان لتلك المعاملات تأثير إيجابي ومعنوي على صفات نمو النبات والإنتاج (الوزن الجاف والرطب لكل من المون المومي لفطر ريزوكتونيا أوريزا، تلاه في ذلك مبيد أميستار توب على فطر يبيولارس سوروكينينا ثم مبيد سكور على الفور الميسليومي لفطر ريزوكتونيا أوريزا، تلاه فعنه]. أبدى المبيد الفطري سكور وأميستار توب فاعلية في تثبيط المو الميسليومي لفطر ريزوكتونيا أوريزا، تلاه في ذلك مبيد أميستار توب على فطر بيبولارس سوروكينينا ثم مبيد سكور على النمو الميسليومي لفطر ريزوكتونيا أوريزا، تلاه في خلي في مولو ولي أعلى فيلم منيو مرارم ولوني المالمي الما مي

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