



APPLICATION OF DATE-PALM WASTE COMPOST FORTIFIED WITH ENDOPHYTIC ELICITORS FOR MANAGEMENT OF POTATO FUNGAL DISEASES

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Abstract

Potatofungal diseases cause significant severe reduction in growth and tubers yield quality. The main object of this study was management of major potato soil borne diseases as black scurf (*Rhizoctonia solani*) and foliar diseases as late blight (*Phytophthora infestans*) and Early blight (*Alternaria solani*) using Date-Palm Waste Compost fortified with endophytic elicitors as *Streptomyces griseus* and *Chaetomium globosium* (1/1). Endophytic elicitors have been found to produce different metabolites and their relationships with their host plants. Two field experiments were conducted at Kalubia Governorate, Egypt, during the two winter growing seasons of 2017/2018 and 2018/2019 to investigate the effect of date-palm compost waste (3 months composted and size 0.5 cm¹) fortified with endophytic elicitors both *S. griseus* and *C. globosium* at rates (5 m³ fed.⁻¹, (feddan = 1.038 acres) on control of fungal diseases of potato plants in compared with commercial composts, chemical fungicides and untreated control. Compared with untreated soil, the palm compost application had a positive impact on all diseases reduction, compared to the control treatment. Compost fortified with *S. griseus* and *C. globosium* significantly reduced all diseases more than used alone of them or individual in both seasons as well as with higher growth and stability. At the same time, palm compost supply significantly increased potato growth and production, during both second seasons. Bio-composting was more effective against fungal diseases, β-1, 3-glucanase activity and chitinase activities of potato plants, polysaccharide-degrading enzyme activities of soil and bio-elicitors as well. Bio compost were the most efficient and might be a promising alternative fungicides for controlling fungal diseases on economic crops. Elicitors of *S. griseus* and *C. globosium* were a highly beneficial endophytic microorganisms for plant protection and growth. The compound is economic and suitable for the environment and waste management.

Key words: Date-Palm Waste Compost, endophytic elicitors, Fungal diseases of Potato, *Streptomyces griseus* and *Chaetomium globosium*.

Introduction

Potatoes (*Solanum tuberosum* L.) are important and most cultivated to reducing worldwide food insufficiency. Fungal soil borne pathogens, including species of *Rhizoctonia*, *Alternaria*, *Fusarium*, *Macrophomina* *Phytophthora*, black scurf caused by *Rhizoctonia solania* and dry rot caused by *Fusarium sambucinum* cause many serious fungal diseases of potato (Agrios, 1997 and Haggag, Wafaa and Abd-El Khair, 2007). In recent years, interests have been generated in the development of alternative fungicides such as bio control agents (Haggag, Wafaa and Abdall 2011), plant resistance inducers PRIs and compost fortified with biocontrol agents to control fungal plant diseases (Haggag, Wafaa and Abd-

El-Kareem 2014). Nowadays, numerous studies have demonstrated that the organic residues, including livestock solid waste, sewage sludge and even green plant waste, after proper composting, could be used with very desirable results as growth media. Utilization of agriculture wastes improve the potential of biocontrol agents in control of disease and soil fertility. Date-Palm extensively exists in the world and produces a lot of waste per annum. Date palm is one of the fruit trees grown in veld ecosystems and produces several wastes during practicing cultivations (Benabderrahim *et al.*, 2018). The use of palm waste cellulose as a substitute for common growing media in *Aglaonema* growing was investigated by Basirat (2011) and showed that palm cellulosid waste could be used as a media individually; and also as an amendment for other media such as sugarcane compost. In nature,

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lignocelluloses are degraded by different microorganisms that produce various hydrolytic enzymes as *Chaetomium* (Abdel-Azeem *et al.*, 2014). Mohammadi-Ghehsareh *et al.*, (2011) showed that date-palm waste could be a media for soilless culture with suitable physical and chemical properties, available and low cost in compare to perlite and coco peat. The date palm trees are normally available in the Northern Africa and in Egypt, each tree produces about 20 kg of dry leaves yearly (Barreveld, 1993). Grinded date palm trees mulch applications provide the additional benefits of decreasing water evaporation from the soil surface, dust suppression, helping prevent soil erosion loss by wind or water, providing thermal stabilization by keeping soil cooler in hot weather and warmer in cool weather. Endophytes elicitors can stimulate plant growth, produce promote secondary metabolites which accumulation in the plant and enhance plant immunity (Haggag, Wafaa 2010 and Murthy *et al.*, 2014). Elicitors of *S.griseus* and *C. globosium* were a highly beneficial endophytic microorganisms for plant protection and have been recognized as an effective biocontrol fungus especially in agriculture which could enhance plant tolerance to biotic and abiotic stresses. The target of this study was examining the effect of using compost Date-palm waste and effective microorganisms as partial replacement of fungicides of potato. The target of this study was management of major potato soil and foliar fungal diseases as dry rot, black scurf, blight using compost date-palm waste fortified with endophytes elicitors *Streptomyces griseus* and *Chaetomium globosium*.

Materials and Methods

Endophytes

The endophytes elicitors of *S. griseus* and *C. globosium* previously isolated from Egyptian soil, identified in Plant Pathology Department, National Research Centre, Egypt. Culture was grown and maintained on solid starch medium (Kuster and Williams, 1964) at 28°C and potato dextrose agar (PDA) medium, respectively. All tested isolates incubated in a rotary shaker at 200 rpm for 72 h at 28 ± 2°C. Cells were harvested by centrifugation at 6,000 rpm for 10 min, washed twice with sterilized water and re-suspended in sterilized distilled water. The concentrations of bacteria cells in the suspensions were adjusted to 1×10⁶ cells/ml and 1×10⁴ cells/ml (cfu/mL) for fungi according to Park, *et al.*, (1991).

Utilization of Date Palm Compost wastes to improvement the potential of endophytes elicitors

Date Palm Compost waste were fortified with endophytes elicitors of *Streptomyces* and *Chaetomium* (1/1), air dried, milled and sieved through a 0.2 mm screen before storing at room temperature prior to usage. All other chemicals used were of the highest purity available and of the analytical grade.

Field Experiments

Utilization of Date-Palm Waste Compost fortified with endophytic elicitors to management of potato diseases

Two field experiments were carried out at private farm at Kalubia Governorate, Egypt, during the two winter growing seasons of 2017/2018 and 2018/2019 to evaluate the efficacy of endophytic elicitors *Streptomyces griseus* and *C. globosium* growing on agricultural date palm wastes in controlling of potato fungal diseases. The applied treatments as follow: biofortified compost, *C. globosium*, *S. griseus*, commercial compost, fungicide Ridomil MZ 72 WP (2.5 kg/ha) and untreated control. *S. griseus* and *C. globosium* were applied as soil application at rate 10 ml/kg compost (3 ton/feddan) 15 days before tuber sowing. A field experiment consisted of plots 4×7 m each comprising of 10 rows and was conducted in completely randomized block design with four plots as replicates for each particular treatment and untreated check. Potato tubers *cv.* Diamond were planted in all treatments. Irrigation and fertilization system were done.

Disease assessment:

Disease incidence (DI) of stem canker was measured as the percentage of plants with a severity rating of 2 or more and the incidence of black scurf was calculated as the percentage of tubers with a severity rating of 1 or more according to the method of Brewer and Larkin (2005). The reduction of the disease incidence and severity was calculated. Early blight disease incidence and severity were estimated and recorded of each treatments. Blight incidence was estimated as the number of infected plants showing disease symptoms in relation to the whole number of potato plants per each plot. Disease severity of early blight incidence was estimated using the disease scale from 0 to 4 suggested by Cohen *et al.*, (1991).

Effects on Plant growth, yield parameters

Efficiency of bio control treatments on vegetative growth, yield and defense enzymes activity of potato plants was evaluated under field conditions.

Plant growth and tuber yield measurements

A sample of 100 plants was randomly taken after 60

days after sowing date, from each experimental plot. Plant growth and yield were measured. Potato yield were recorded after harvesting (kg/plot).

Enzymatic activity

Determination of β -1,3-glucanase activity and chitinase activities of potato plants:

Potato plants after 60 days of sowing were used as samples to determine β -1, 3-glucanase activity and chitinase activity.

Extraction of enzymes:

Potato leaves (1.0 g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.1) at the rate of 1/3 w/v, centrifuged at 3000 rpm for 15 min, then, supernatant was used to determine enzyme activity.

β -1, 3-glucanase assay:

β -1, 3-glucanase activity was determined with the method of Abeles and Forrence (1970). Laminarin was used as substrate and dinitrosalicylic acid as reagent to measure reducing sugars. The reaction was stopped by adding 1 mL of dinitrosalicylic acid reagent and heating the tubes for 5 min at 100°C with optical density at 500 nm. The β -1,3-glucanase activity was expressed as millimolar glucose equivalent released/gram fresh weight tissues/60 min.

Extraction of chitinase enzyme:

Chitinase activity was determined after 60 days of planting and the enzyme extraction was done according to method of Tuzun *et al.*, (1989).

Chitinase assay:

Chitinase activity was measured by colourimetric method of Boller and Mauch, (1988). Colloidal chitin was used as a substrate and dinitrosalicylic acid as reagent to measure reducing sugars. Chitinase activity was expressed as mM N-acetylglucose amine equivalent released/gram fresh weight tissue/60 minutes.

Enzyme activity assays of soil:

Polysaccharide-degrading enzyme activities of soil treated with bio and or compost activity were analyzed using the 3, 5-dinitrosalicylic acid (DNS) method by measuring the amount of reducing sugars liberated (Miller 1959) at 540 nm using a UV-Vis spectrophotometer. One enzyme activity unit (U) is defined as the amount of enzyme required to release 1 μ mol of reducing sugars from a substrate in 1 min

under the assay condition.

Bioassay of endophytic elicitors activities in soil:

Isolation and enumeration of endophytic elicitors in soil rhizosphere of potato plants in harvest stage were performed using serial dilution agar plate technique (Ahmad *et al.*, 2017) using Starch nitrate agar and Potato Dextrose agar the plates were incubated at 25°C for 7 days.

Data Analysis

Data were analyzed using ANOVA of square-transformed data. Significant differences were assessed by comparison with the differences between means using LSD value at 0.05.

Results

Utilization of Date-Palm Waste Compost fortified with endophytic elicitors to management of potato diseases

The efficiency of endophytic elicitors *S.griseus* and *C. globosum* alone or in co-cultures of Date-Palm Waste Compost in management of potato diseases compared with under field was measured during 2017-2018/2018-2019 seasons. Results in table 1 indicated that the efficacy of Date-Palm Waste Compost fortified with endophytic elicitors showed superior effect in controlling of black scurf and stem canker diseases caused by *Rhizoctonia solani*, in compared with endophytic elicitors, agricultural wastes and fungicide.

Results in table 2 indicated that the highest reduction of early blight disease severity of potato plants was

Table 1: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost in management of potato black scurf and stem canker diseases under field conditions.

Treatment	Reduction %			
	Black scurf		Stem canker	
	Season 2017/2018	Season 2018/2019	Season 2017/2018	Season 2018/2019
<i>S. griseus</i>	88.0cd*	86.9cd	88.8cd	87.9cd
<i>C. globosum</i>	87.0cd	87.9cd	87.4cd	87.1cd
<i>S. griseus/C. globosum</i>	89.4c	88.8c	90.7c	90.4c
<i>S. griseus</i> /Compost	95.2b	95.7b	94.8b	95.2b
<i>C. globosum</i> /Compost	96.4ab	97.5ab	95.4ab	96.8ab
<i>S. griseus/C. globosum</i> + Compost	98.4a	99.8a	98.7a	99.6a
Compost	79.8e	79.7e	80.8e	81.0e
Fungicide Ridomil –MZ	88.0cd	87.9cd	88.4cd	88.1cd
Control	74.3f	76.5f	78.0f	78.4f

*Values with the same letter are not significantly different ($p = 0.05$).

Table 2: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost in management of potato early and late blights diseases severity under field conditions.

Treatment	Early blight (<i>Alternaria solani</i>)		Late blight (<i>Phytophthora infestans</i>)	
	Season 2017/2018	Season 2018/2019	Season 2017/2018	Season 2018/2019
	<i>S. griseus</i>	3.5 b*	3.7 b	1.9 bc
<i>C. globosum</i>	3.7 b	3.9 b	2.2 b	2.6 b
<i>S. griseus/ C. globosum</i>	3.3 bc	3.4 bc	1.8 bc	2.3 bc
<i>S. griseus</i> /Compost	2.8 b	2.9 b	1.9 b	2.2 b
<i>C. globosum</i> / Compost	3.3 bc	3.6 bc	1.6 c	1.9 c
<i>S. griseus/ C. globosum</i> + Compost	2.3 cd	2.6 cd	1.3 d	1.6 d
Compost	3.7 b	3.8 b	2.2 b	2.6 b
Fungicide Ridomil –MZ	1.3 d	1.5 d	1.8 bc	2.3bc
Control	7.3 a	7.8 a	5.3 a	6.3 a

*Values with the same letter are not significantly different (p = 0.05).

obtained with *S. griseus* and *C. globosum* of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost as compared with single treatments and untreated control and fungicide. Also, treated plant with *S. griseus* and *C. globosum* were significantly reduced the disease severity of early blight on both seasons. The same data was found with late blight diseases that the highest reduction was obtained with *S. griseus* and *C. globosum* of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost as compared with single treatments and untreated control.

Effects on vegetative growth and yield of potato

Data in table 3 indicated that the applied endophytic

Table 3: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost in Potato plant growth and tuber yield grown under field conditions during two seasons 2018 and 2019.

Treatment	Season 2017/2018		Season 2018/2019	
	Dry weight (g./plant)	Av. tuber yield kg/plot	Dry weight (g./plant)	Av. tuber yield kg/plot
<i>S. griseus</i>	77.4b*	8.3b	79.1b	8.9b
<i>C. globosum</i>	78.8b	7.9b	78.1b	7.1b
<i>S. griseus/ C. globosum</i>	90.9ab	10.2a	91.4ab	10.7a
<i>S. griseus</i> /Compost	88.7ab	8.3b	88.1ab	8.7b
<i>C. globosum</i> / Compost	89.3ab	9.1ab	80.2ab	9.8ab
<i>S. griseus/ C. globosum</i> + Compost	118.7a	12.3a	116.6a	13.0a
Compost	67.8c	6.9c	69.7c	7.1c
Fungicide Ridomil –MZ	55.7d	5.8d	57.1d	5.6d

*Values with the same letter are not significantly different (p = 0.05).

elicitors *S. griseus* and *C. globosum* alone or in co-cultures of Date-Palm Waste Compost resulted in significant increasing in vegetative dry weight and tubers yield during both seasons. Since, treated plants was *S. griseus* and *C. globosum* grown on Date-Palm Waste Compost give the same effective of fungicides (Redomil –MZ). Also, treated potato plants with *S. griseus* and *C. globosum* in co-cultures of Date-Palm Waste Compost resulted in considerable increasing dry weight/plant and yield kg/plot in two growing seasons in compared with single treatments and untreated control.

Effects on chitinase and β-1, 3-Gluconase enzymatic activities:

The efficiency of applied endophytic elicitors *S. griseus* and *C. globosum* grown in Date-Palm Waste Compost individually or in co cultures on chitinase and β-1, 3-Gluconase enzymatic activities in treated potato plants was investigated. Results in Fig. 1 indicate that *S. griseus* and *C. globosum* were effective in increasing enzymatic activity of chitinase in treated plants compared with non-treated plants. The most effective treatments were observed in *S. griseus* and *C. globosum* fortified with Date-Palm Waste Compost compared with untreated plants during in both seasons and single treatments. The same results also were recorded in Fig. 1 that *S. griseus* and *C. globosum* were effective in increasing β-1, 3-Gluconase enzymatic activity in treated plants compared with non-treated plants. The

most effective treatments were observed in *S. griseus* and *C. globosum* fortified with Date-Palm Waste Compost compared with untreated plants during in both seasons and single treatments.

Effects on Polysaccharide-degrading enzyme and endophytic elicitors activities of soil:

The efficiency of applied endophytic elicitors *S. griseus* and *C. globosum* grown in Date-Palm Waste Compost individually or in co cultures on Polysaccharide-degrading enzyme activities in soil was investigated. Results in Fig. 2 found that *S. griseus* and *C. globosum* were more effective in increasing Polysaccharide-degrading enzyme activity in treated soil compared with non-treated plants. The most effective treatments were observed in *S. griseus/C. globosum* fortified with Date-Palm Waste Compost compared with untreated plants during in both seasons and single

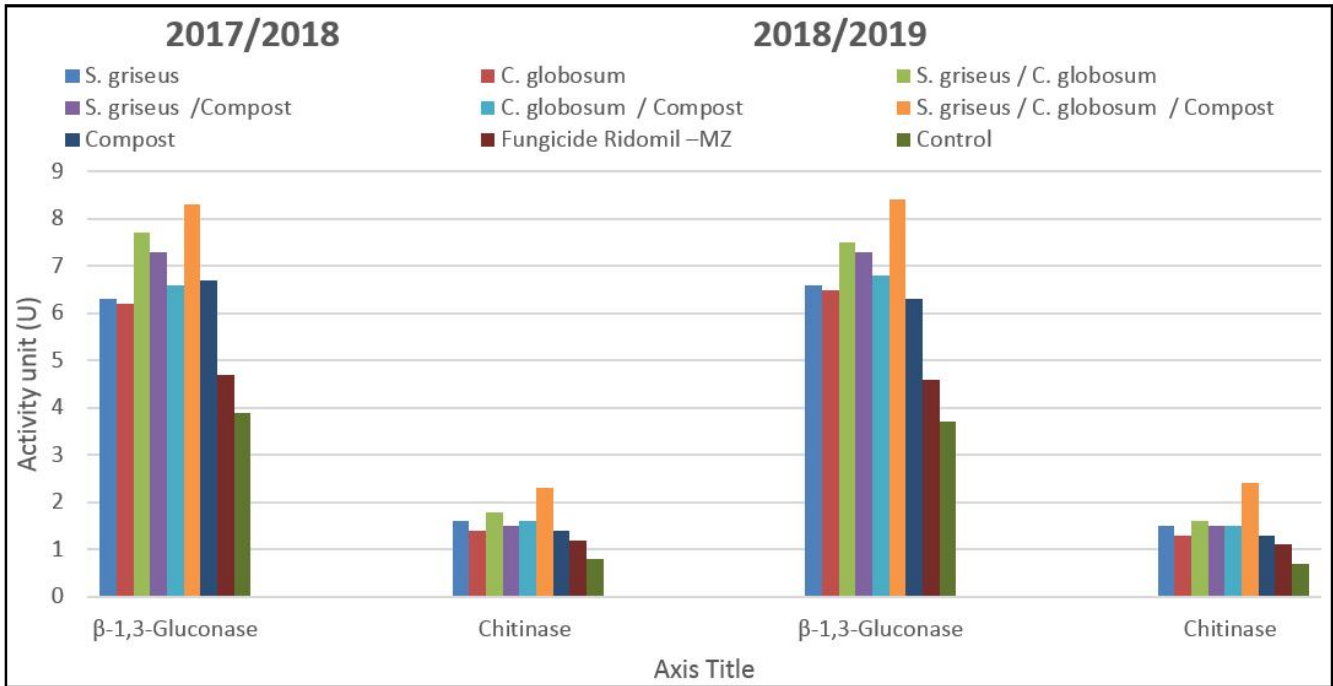


Fig. 1: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost on β-1, 3-Gluconase and Chitinase enzymes activities in potato plants grown under field conditions.

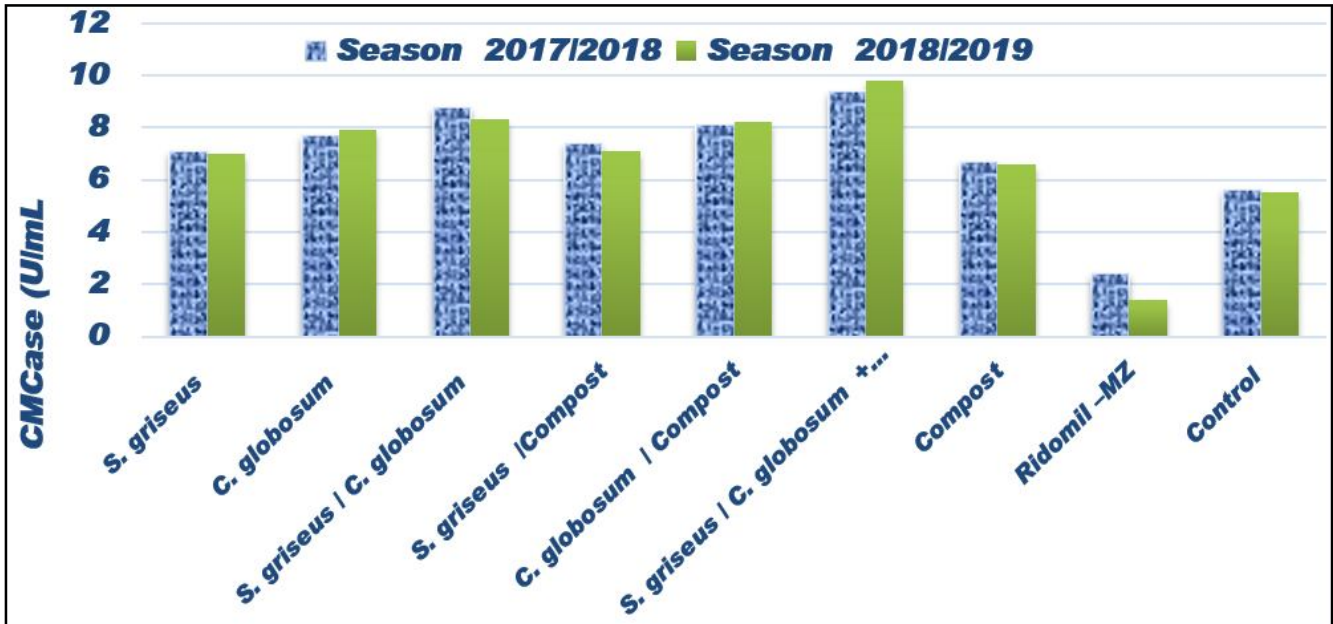


Fig. 2: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* co-cultures of Date-Palm Waste Compost on Polysaccharide-degrading enzyme in treated soil under field conditions.

treatments.

The results also were recorded in table 4 that *S. griseus* and *C. globosum* were increasing in soil treated plants compared with Date-Palm Waste Compost in compared with untreated plants during both seasons and single treatments.

Discussion

In the present research it was demonstrated that the

using of date palm waste composts fortified with endophytic elicitors *S. griseus* and *C. globosum* effectively protect potato from plant phytopathogenic fungi and improve crop productivity. Since, under field conditions, soil borne diseases as black scurf (*Rhizoctonia solani*) and foliar diseases as late blight (*Phytophthora infestans*) and Early blight (*Alternaria solani*). Furthermore, controlling of *Rhizoctonia* diseases of potato was achieved using Composted Date-Palm

Table 4: Efficiency of endophytic elicitors *S. griseus* and *C. globosum* in co-cultures of Date-Palm Waste Compost on endophytic elicitors activity in soil under field conditions.

Treatment	Endophytic elicitors activity			
	Season 2017/2018		Season 2018/2019	
	<i>S. griseus</i>	<i>C. globosum</i>	<i>S. griseus</i>	<i>C. globosum</i>
<i>S. griseus</i>	2×10 ⁶	0	2×10 ⁶	0
<i>C. globosum</i>	0	2.4×10 ⁴	0	2×10 ⁴
<i>S. griseus/C. globosum</i>	3.4×10 ⁶	0	3.3×10 ⁶	0
<i>S. griseus</i> /Compost	5.5×10 ⁶	0	6×10 ⁶	0
<i>C. globosum</i> /Compost	0	11.2×10 ⁴	0	11×10 ⁴
<i>S. griseus/C. globosum</i> + Compost	11.0×10 ⁶	14.3×10 ⁴	11.3×10 ⁶	15×10 ⁴
Compost	ND		ND	
Ridomil –MZ	ND		ND	
Control	ND		ND	

Waste fortified with *C. globosum* or *S. griseus*.

The beneficial effects of using date palm compost on growth and fruiting of potato might be attributed to its essential roles on enhancing both physical and chemical characteristics of the soil, vitamins enzymes and antibiotics as well as root development, availability of most nutrients, water retention, soil aggregation and soil exchange capacity (Tuzun *et al.*, 1989, Mohammadi *et al.*, 2011 and Benabderrahim *et al.*, 2018). A lignocellulose-degrading enzyme activity by *Chaetomium globosum* was characterized and modification in bio-industries (Wanmolee *et al.*, 2016). Previous studies showed that using organic manures were very effective in improving growth aspects, nutritional status of the trees, yield and both physical and chemical characteristics of the fruits in different crops. Many investigators that, the role of endophytic microorganisms as *S. griseus* and *C. globosum* induce host defense responses in many plants against many pathogens (Haggag, Wafaa 2010 Haggag, Wafaa and Abdall, 2011 and Ahmad *et al.*, 2017).

Endophytic microorganisms special biocontrol agents are known to reduce disease severity and improve plant productivity and soil health. The use of biological methods are one of the safest and most successful processes, since microorganisms may play an important biological role, function and restore the balance within the environmental system and convert the date palm wastes into products. *C. globosum* known to produce cellulolytic enzyme systems which may depend on the nature of substrates (Wanmolee *et al.*, 2016). Haggag Wafaa *et al.*, (2012) demonstrated that an industrial enzyme chitinase are produced by endophytic *Streptomyces* and have antifungal activity against phytopathogens as *Rhizoctonia solani*, *Fusarium oxysporum*, *Alternaria alternata* and

Phytophthora parasitica in media containing chitin.

In conclusion, wastes of date palm trees might be recycled biologically into organic products that have use as biofungicides. The using of date palm waste composts fortified with endophytic elicitors *S. griseus* and *C. globosum* are more effectively in protect plant from phytopathogenic fungi and improve crop productivity. *Chaetomium globosum* was a highly beneficial endophytic microorganism for the have antifungal activity and metabolism production.

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