Improvement in potential of enzymes from Chaetomium globosum and Trichoderma harzianum using different agricultural wastes and its applications.

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Different agricultural wastes were used as cheapest and most economic substrates for enzymes production from Chaetomium globosum and Trichoderma harzianum. Five different fungal isolates of C. globosum and T. harzianum were tested for their antagonistic effect against Alternaria solani, Th4 & Ch2 was the most promising and were used for production of hydrolytic enzymes (endo-GN; EC 3.2.1.4.) and endopolymethyl-galacuronase (endo-PMG; EC3.2.1.15) as free/co-cultured using 60 g/l soy bean (Sb) as carbon source. The co-cultured Th4 & Ch2 grown on Sb were more promising in reduction of early blight of potato, increasing in vegetative growth as well as tubers yield of treated potato plants during 2017 and 2018 seasons. The efficiency of applied biological treatment of Th4 & Ch2 grown on 60 g/l Sb individually or in co cultures on chitinase and β-1, 3-gluconase enzymatic activities in treated and non-treated potato plants increased enzymatic activity in treated plants compared with non-treated plants.

Keywords: Agricultural wastes, fungi, hydrolytic enzymes, chitinase, β-1, 3-gluconase, bicontrol, antagonistic effects.

INTRODUCTION

Addition of agro-industrial wastes can be used as substrate or composition of media for enzymes production by different microbial genus. The use of agro-industrial wastes have environmental and economic benefits due to their reduction of costs of enzymes production, their low commercial value and applications of these residues (Carlos et al., 2014).

In recent years, there has been an increasing trend towards value addition of agro-industrial residues and efficient utilization such as cassava husk, coffee pulp, sugarcane bagasse, apple pomace, sugar beet pulp, declassified potatoes, corn stover, corn cob, barley straw, wheat straw, rice straw, sugarcane bagasse, sorghum husks, coconut husks, pineapple peel, switch grass and banana leaves (Demain, 2005; Soccol and Vandenberghhe 2003).

Microorganisms able to degrade lignocellulosic materials which affected by some factors, namely crystallinity of cellulosic fiber and porosity of the wastes materials (Zhang et al., 2006). The molecular organization of the plant fiber cell wall which is the lignocellulose complex composed mainly of cellulose, hemicellulose, and lignin which limits the biodegradation of this complex (Lo et al., 2008). Pretreatment of this wastes by fungi increases the surface area of the lignocellulosic materials, reduce crystallinity of cellulose and ultimately improves the biodegradation (Kumar et al., 2008).
Endoglucanase and Endo-polymethyl-galacturonase both hydrolytic enzymes which considerable commercial value produced by fungi and bacteria. Endoglucanase used in the food industry, for baking and fruit and vegetable processing, breakdown of agricultural wastes, in the manufacture of animal feed, e.g., a mono-gastric animal feed, such as a swine or poultry (e.g., chicken) feed, in pulp and paper production, textile manufacture, household, industrial cleaning agents. Endoglucanases are also important for the digestion of cellulose, a beta- 1,4-linked glucan found in all plant material. Endopolymethyl-galacturonase hydrolyzes the a-1, 4 glycosidic bonds of non-esterifies portions of pectic substrates which are major components of plant cell walls (Robert et al., 2013).

Many species of Chaetomium with the potential as biological control agents suppress the growth of bacteria and fungi through mycoparasitism, competition, antibiosis or their various combinations. Some species of Chaetomium have been reported to work as antagonists against several plant pathogens (Zhang and Yang 2007; Charoenporn et al., 2010; Sibounnavong et al., 2012& Shanthiyaia et al., 2013).

The objective of this research was the use of different agricultural wastes for production of hydrolytic enzyme for further determinations of endoglucanase (endo-GN; EC 3.2.1.4.) and endopolymethyl-galacturonase (endo-PMG; EC3.2.1.15) by Trichoderma harzianum and Chaetomium globosum and to evaluate the effects of growing and bio control agents on agricultural wastes for inhibition of Alternaria solani inhibition. In vitro, suppression of early blight disease as well as plant growth, enzymes activities and yield parameters under field conditions.

**MATERIALS AND METHODS**

**Microorganisms:**

**Source of pathogen, bio control agents isolates and potato tubers:**

Five different fungal isolates of Chaetomium globosum (Ch1,Ch2,Ch3,Ch4&Ch5) and Trichoderma harzianum (Th1,Th2,Th3, Th4 &Th5) were isolated from soil samples collected from rhizosphere of plants using serial dilution technique (Soytong, 1989).

The pathogenic isolate of Alternaria solani was isolated from the potato leaves showing typical symptoms of early blight. All isolates were identified according to Simmsmons (2007).

The cultures were maintained on potato dextrose agar medium Ainsworth, 1961) for further studies. Potato tubers (Solanum tuberosum L.) cv. Diamond obtained from Vegetables Crop Research Department, Agricultural Research Centre, Giza, Egypt were used in the present study.

**Fungicide:**

The fungicide Ridomil MZ 72 WP produced by Syngenta, Bazil, Switzerland contains: metalaxyl 8% (fenylamole) and mancozeb 64% (dithiocarbamate complex) with zink salt was applied as comparative treatment.

**Agricultural wastes materials:**

Agricultural wastes such as sugarcane bagasse (Sb), rice straw (Rs), soy bean (Sb) and sawdust (S) were chosen as the carbon substrates in this study. The agricultural wastes materials were 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.

**In vitro: Antagonistic studies between T. harzianum and C. globosum against pathogenic microorganisms:**

The antagonistic activity of five isolates from T. harzianum (Th1, Th2, Th3, and Th4&Th5) and C. globosum (Ch1, Ch2, Ch3, Ch4 & Ch5) against A. solani were evaluated. Five mm actively growing mycelial disc of A. solani was placed on PDA medium 1.0 cm away from the edge of the petri plates compared with five mm culture disc of each C. globosum and for T. harzianum isolates were placed on the same petri dish. The plates were incubated at room temperature (27°C) for 7 days. The inhibition of mycelial growth of A. solani was measured in millimeters when A. solani had fully grown (9 cm) over PDA in petri-plates control check treatment. Four replications were maintained for each bio control agent isolates (Anees et al., 2010).

**Measurement of sporulation:**

Sporulation was studied at the end of incubation period, 6mm in diameter agar discs were cut from the margin of the colony in each treatment and transferred to a vial containing 10 ml of sterile distilled water, the suspension was continuously shaken for 5 min, after which time, the density of spores/ml was counted by a haemo-
cytometer according to (El- Abyad et al., 1983). Four plates were used for each treatment and the mean number of spores was calculated. The isolates which showed higher inhibition were selected and used for further studies.

Fermentation media:
The culture media was composed of (g/l): NaNO\textsubscript{3} 2.0, K\textsubscript{2}HPO\textsubscript{4} 0.5, KCl 0.5, MgSO\textsubscript{4}.7H\textsubscript{2}O 0.5, sucrose was replaced by 20 g/l of selected agricultural wastes. Two discs (6 mm in diameter) from 7 days old cultures of Chaetomium globosum (Ch) and Trichoderma harzianum (Th) were transferred to 250 ml Erlenmeyer conical flasks each containing 50 ml fermentation medium. The inoculated flasks were incubated on a rotary incubator shaker at 180 r.p.m for 7 days at (28-30° C). At the end of incubation period, cultures were centrifuged at 8000 r.p.m. The cell free supernatant was used as a crude hydrolytic enzyme for further determinations of endoglucanase (endo-GN; EC 3.2.1.4.) and endopolymethyl-galacturonase (endo-PMG; EC3.2.1.15).

Enzymes assays:

1-Hydrolytic enzymes:
All hydrolytic enzymes were assayed according to Miller (1959) using carboxy-methyl cellulose for endoglucanase (endo-GN; EC 3.2.1.4.) and citrus pectin for endopolymethyl-galacturonase (endo-PMG; EC3.2.1.15).

The reaction mixture contained 1 ml of 0.5 % substrate in 50 mM citrate phosphate buffer pH (4.8) with 0.2 ml enzyme , incubate at 50° C for 30 min , then add 1ml of DNS ,reducing sugar was measured at 540 nm. One unit of enzyme activity was defined as the amount of enzyme that converts one micromole of reducing sugar per minute reaction under the described condition.

2-Determination of β-1, 3-glucanase activity and chitinase activities:
Potato plants after 60 days of sowing were used as samples to determine β-1, 3-glucanase activity and chitinase activity done according to method of (Tuzun et al., 1989).

Extraction of enzymes from plant leaves:
Plant leaves (1.0 g) were homogenized with 0.1 M sodium phosphate buffer (pH 7.0) (Goldschmidt et al., 1966) at the rate of 1/3 w/v. The homogenate was centrifuged at 3000 rpm for 15 min. The supernatant was used to determine β-1, 3-glucanase activity and chitinase activities.

2. a.β-1, 3-glucanase assay:
The method of (Abeles and Forrence 1970) was used to determine β-1, 3-glucanase activity. Laminarin was used as substrate and dinitrosalicylic acid (DNS) as reagent to measure reducing sugars. The method was carried out as 0.5 ml of enzyme extract was added to 0.5 ml of 0.05 M of potassium acetate buffer (pH 5.0) containing 2% laminarin. The mixture was incubated at 40°C for 60 min. The reaction was stopped by adding 1 ml of DNS reagent and heating the tubes for 5 min at 100°C. The tubes were cooled then 3 m of distilled water were added before assay. The optical density was measured at 500 nm. One unit of β-1, 3-glucanase activity was expressed as millimolar glucose equivalent released/gram fresh weight tissues/60 min.

2. B. Chitinase assay:
Chitinase activity was determined by colorimetric method of (Boller and Mauch1988). 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.

Optimization conditions of hydrolytic enzymes production:

Effect of mixed and free cultures on enzymes production by fungi:
The two selected fungal isolates T. harzianum (Th4) and C. globosum(Ch2)were inoculated in fermentation medium to compare the efficacies of mixed and co-culture of the isolates on enzymes production as mentioned above.

Effect of different concentrations of agricultural wastes on enzymes production:
Different weights of soy bean(Sb) (20-80 g/l) were tested for enzymes production in fermentation medium inoculated with co-culture of T. harzianum (Th4) and C. globosum (Ch2) after 7 days incubation period at 200r.p.m

Field experiment:

Effects of bio agents on early blight disease severity under field conditions:
The field experiments were carried out at private farm at El-Kanater , Qalubia governorate
during 2017 and 2018 growing seasons to evaluate the efficacy of *C. globosum* (Ch2) and *T. harzianum* (Th4) growing 60 g/l soy bean (Sb) in controlling early blight of potato plants under natural field conditions. The applied treatments as follows:

2. *Chaetomium globosum* (Ch2).
3. Th4 + (Ch2).
4. Th4 + Sb
5. Ch 2 + Sb
6. (Th4+ Sb) + (Ch2 + Sb)
7. Fungicide
8. Control

All treatments were used as tuber treatment and soil applications as well as plant foliar spray (at 4–5 true leaf stage) three times with 15 days interval. The fungicide Ridomil MZ 72 WP (2.5 kg/ha) was used in this study as a comparison. The liquid formulations of Th4 + AW and (Ch2 + AW) were applied as tuber treatment, soil applications and foliar spray in integrative treatments. Potato tubers were soaked in liquid formulation of each bio agent treatments water for 30 min before sowing. The soil application was given at 30 days after sowing. The liquid formulation was added at 50 ml / kg of plant compost (5 ton/feddan) 15 days before tuber sowing.

A field experiment consisted of plots 3 x 7 m each comprising of 7 rows and 15 holes/row and was conducted in completely randomized block design with four plots as replicates for each particular treatment and untreated check. Potato tubers cv. Diamond was planted in all treatments. All plots received traditional agricultural practices such as irrigation and fertilization. Early blight disease incidence and severity were estimated and recorded of each treatment.

**Disease assessment:**

Early 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) as follows: 0 = no leaf lesion; 1= lesions on < 25% of leaf area; 2 = lesion on 26–50% of leaf area; 3 = lesion on 51–75% of leaf area and 4 = lesions on 76 up to 100% of leaf area. Then the following formula was used

\[ DS = \sum (CXN) / N \]

Where:

Note: DS = Disease severity, n = Number of infected plants per category, c = Category number and N = Total examined plants.

**Evaluation of plant growth, yield parameters and enzymatic activity:**

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

**Plant growth measurements:**

A sample of 25 plants was randomly taken at flowering stage (60 days after sowing date), from each experimental plot. Plant growth parameters such as plant height (cm) and the number of leaves per plant were recorded.

**Determination of tuber yield:**

At harvesting stage (90 days after sowing), total yield from each plot were harvested and the weight of tubers (kg/plot) as well as increasing in tuber yield over the control treatments were calculated.

**Statistical analysis:**

One way analysis of variance (ANOVA) was used to analyze differences between treatments. MSTAT-C program (V2.1) was used to perform the analysis of variance of treatments. Duncan’s Multiple Range Test was used for means separation (Neler *et al.*, 1985).

**RESULTS AND DISCUSSION**

**Antagonistic activity of *C. globosum* (Ch) and *T. harzianum* (Th) against *A. solani.*

Five different fungal isolates of *Chaetomium globosum* (Ch1, Ch2, Ch3, Ch4 & Ch5) and *Trichoderma harzianum* (Th1, Th2, Th3, Th4 & Th5) were evaluated for their antagonistic effects against *A. solani.*
Table (1): Antagonistic activity of different isolates of *C. globosum* (Ch) and *T. harzianum* (Th) against *A. solani* on PDA medium.

<table>
<thead>
<tr>
<th>Bio agents</th>
<th>Av. Linear Growth (mm)</th>
<th>Reduction %</th>
<th>Av. spores production</th>
<th>Reduction %</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Th1)</td>
<td>26 d</td>
<td>71.1</td>
<td>10.2dd</td>
<td>68.4</td>
</tr>
<tr>
<td>(Th2)</td>
<td>31 c</td>
<td>65.6</td>
<td>12.8c</td>
<td>60.0</td>
</tr>
<tr>
<td>(Th3)</td>
<td>33 c</td>
<td>63.3</td>
<td>13.4c</td>
<td>58.0</td>
</tr>
<tr>
<td>(Th4)</td>
<td>18 e</td>
<td>80.0</td>
<td>8.8 e</td>
<td>72.4</td>
</tr>
<tr>
<td>(Th5)</td>
<td>26 d</td>
<td>71.1</td>
<td>10.8d</td>
<td>66.2</td>
</tr>
<tr>
<td>(Ch1)</td>
<td>30 c</td>
<td>66.6</td>
<td>12.8d</td>
<td>60.0</td>
</tr>
<tr>
<td>(Ch2)</td>
<td>25 d</td>
<td>72.2</td>
<td>10.8d</td>
<td>66.2</td>
</tr>
<tr>
<td>(Ch3)</td>
<td>38 b</td>
<td>57.8</td>
<td>15.4b</td>
<td>52.8</td>
</tr>
<tr>
<td>(Ch4)</td>
<td>32 c</td>
<td>64.4</td>
<td>12.2c</td>
<td>62.0</td>
</tr>
<tr>
<td>(Ch5)</td>
<td>40 b</td>
<td>55.5</td>
<td>15.8b</td>
<td>50.8</td>
</tr>
<tr>
<td>Control</td>
<td>90 a</td>
<td>0.0</td>
<td>32x106 a</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Results in Table (1) showed that all five isolates of *T. harzianum* and *C. globosum* were found to inhibit the colony growth and spore production of early blight pathogen *A. solani* in vitro, however, *T. harzianum* isolateTh4 showed higher inhibition to *A. solani* followed by Ch2 and Th1. The percent reduction in both liner growth and spores production of *A. solani* in vitro was significantly higher in Th4 (80.0% and 72.4%) , Ch 2 (72.2% and 66.2%) when compared to other isolates Th3 (63.3 and 58.0%), Th2 (65.6 and 60.0 %), Ch5 (55.5 and 50.8%) and Ch1 (66.6 and 60.0%). In this respect many species of *C. globosum* with the potential as biological control agents suppress the growth of bacteria and fungi through competition, mycoparasitism, antibiosis, or their various combinations. These results are agreement with finding reported with (Zhang and Yang 2007; Charoenporn et al., 2010 ; Zhang et al., 2012;Sibounnavong et al., 2012 ; Shanthiyaa et al., 2013). They noted that some species of *C. globosum* have been reported to work as antagonists against several plant pathogens, such species of *C. globosum* suppress the growth of bacteria and fungi through competition, mycoparasitism, antibiosis, or their various combinations. In addition, *Chaetomium* species are known as producers of many different bioactive metabolites which play important roles in their biological control activities. One *Chaetomium* species may produce many metabolites with different bioactivities and molecular weight, this lead to differences in antifungal activity of its crude extracts (Kanokmedhakul et al., 2007). The maximum inhibition of *Alternaria porri* (Ellis) Cif. was recorded in *T. harzianum* with 79.5% inhibition)(Chethana et al., 2012). The antagonistic effects of species of *Trichoderma* against pathogen might be due to (a) production of non-volatile toxic substances diffuse in the substrate and (b) parasitism by the antagonists. The formation of inhibition zone indicates the production of antibiotics by the antagonists which diffused in the medium.

### Enzymes production:

#### Effect of free and co-cultured cultures on enzymes production by fungi:

From the above result *T. harzianum* (Th4) and *C. globosum* (Ch2) were the most promising isolates for inhibition of *A. solani* and were tested for enzymes production in fermentation media containing Sb, Rs, S & Aw incubated for 7 days at 28-30°C 200 r.p.m.

Results in Table (2) showed that endoglucanase and endopolymer methyl-galacturonase produced were at their maximum activities while using co-cultured of (Th4) and (Ch2) on soy bean (Sb) as carbon source produce (8.87 & 5.50 U/ml) respectively. By comparison by other wastes enzymes production increase using the same waste soy bean (Sb) inoculated by (Th4) produce (5.00 U/ml) for both enzymes. Culture filtrate inoculated with *C. globosum* (Ch2) produce maximum activity using (Sb) as carbon source for endoglucanase (5.87 U/ml) while sawdust (S) was most suitable for endopolymer methyl-galacturonase produce (4.35 U/ml). From all the above results co-culture were more suitable for both enzymes production. These results were coincided with (Hag et al., 2005) who found that co- cultures of *A. niger* and *T. viride* were most suitable for enzymes production.
Table (2): Effect of free and co-cultured cultures on enzymes production by using different agricultural wastes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Hydrolytic enzymes activity (U/ml)</th>
<th>Endoglucanase</th>
<th>Endopomethyl-galacturonase</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T. harzianum (Th4)</strong></td>
<td></td>
<td>5.76</td>
<td>4.02</td>
</tr>
<tr>
<td>Th4 + (Sb)</td>
<td></td>
<td>4.08</td>
<td>4.50</td>
</tr>
<tr>
<td>Th4 + (Rs)</td>
<td></td>
<td>3.65</td>
<td>3.15</td>
</tr>
<tr>
<td>Th4 + (Sb)</td>
<td></td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Th4+ (S)</td>
<td></td>
<td>3.08</td>
<td>2.69</td>
</tr>
<tr>
<td>Th4+ (mixed wastes AW)</td>
<td></td>
<td>3.04</td>
<td>3.36</td>
</tr>
<tr>
<td><strong>C. globosum (Ch2)</strong></td>
<td></td>
<td>2.60</td>
<td>1.21</td>
</tr>
<tr>
<td>Ch2 (Sb)</td>
<td></td>
<td>2.47</td>
<td>4.71</td>
</tr>
<tr>
<td>Ch2 (Rs)</td>
<td></td>
<td>3.00</td>
<td>3.00</td>
</tr>
<tr>
<td>Ch2 (Sb)</td>
<td></td>
<td>5.87</td>
<td>3.35</td>
</tr>
<tr>
<td>Ch(S)</td>
<td></td>
<td>1.75</td>
<td>4.35</td>
</tr>
<tr>
<td>Ch2 (mixed wastes AW)</td>
<td></td>
<td>4.87</td>
<td>3.00</td>
</tr>
<tr>
<td>Th4+ Ch2</td>
<td></td>
<td>3.52</td>
<td>3.21</td>
</tr>
<tr>
<td>Th4 + Ch2 +(Sb)</td>
<td></td>
<td>3.73</td>
<td>1.95</td>
</tr>
<tr>
<td>Th4 + Ch2 +(Rs)</td>
<td></td>
<td>3.52</td>
<td>0.37</td>
</tr>
<tr>
<td>Th4 + Ch2 +(Sb)</td>
<td></td>
<td>8.87</td>
<td>5.50</td>
</tr>
<tr>
<td>Th4+ Ch2 +(S)</td>
<td></td>
<td>3.39</td>
<td>1.47</td>
</tr>
<tr>
<td>Th4 + Ch2 +(AW)</td>
<td></td>
<td>5.21</td>
<td>2.38</td>
</tr>
</tbody>
</table>

Enzymes production was high with mono strain of *A. niger* and enzymes decreased with mixed fungal strains (Hernandez et al., 2014). Co-culturing of *Candida bombicola* and *Hypocrea jecorina* Rut C30 acts as inducer for enzyme production (Lo and Ju, 2009).

**Effect of different concentrations of soy bean on enzymes production:**

Different weights of soy bean (Sb) (20-80 g/l) were tested for enzymes production in fermentation medium inoculated with co-culture of *T. harzianum* (Th4) and *C. globosum* (Ch2) after 7 days incubation period at 200 r.p.m. Results in Fig (1) showed that 60 g/l was most effective for endoglucanase production produce 19.04 U/ml as well as 60 g/l was effective for endopomethyl-galacturonase produce 21.76 U/ml. 10 g/l of ground nut produce maximum enzymes activity in co cultures of *A. terreus* and *T. viride* (Asish and Deepek, 2005). Production of enzymes in fermentation medium inoculated by co –cultures of *A. niger* and *T. viride* using 20 g/l of sugar cane bagasse and wheat bran (Muhammad et al., 2013).

**Management of early blight disease under field conditions:**

The efficiency of growing *C. globosum* (Ch2) and *T. harzianum* (TH4) alone or in co-cultures on 60 g/l soy bean (Sb) as well as the fungicides (Redomil – plus at 2 g / l ) as comparative treatment in control of early blight disease of potato plants as well as the effects on plant growth (plant high and number of leaves) and tuber yield of potato plants under field was evaluated during 2017 and 2018 seasons.

**Effect on early blight disease severity:**

Results in Table (3) showed that single or integrative treatments of bio control agents *T. harzianum* (TH4) and *C. globosum* (Ch2) grow on 60 g/l of soy bean as carbon source were significantly reduced the disease severity of early blight on potato plants during 2017 and 2018 seasons. The highest reduction records of disease severity was obtained when the integrative treatments of Th4 + Ch2 grown on agricultural wastes (AW) and Th4 + Ch2 grown on PDA broth as compared with single treatments and untreated plants (control). The most effective treatments were the mixture of Th4 + Ch2 +AW and Th4+ Ch2 followed by fungicides (Redomil – MZ1), they suppressed early blight on potato by (75.0, 69.4, 64.3 and 61.1)% during 2017 and 2018 seasons respectively. In this respect, (Shanthiyaa et al., 2013) noted that the application of *C. globosum* resulted in greater potato tuber yield by reducing late blight infection during two field trials when compared to untreated controls.
El Gamal et al., Improvement in potential of enzymes

Figure (1): Effect of different concentrations of soy bean.

Table (3): Potato early bight disease severity in response to different bio control agents and fungicide treatments during two seasons 2017/2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season 2017</th>
<th>Season 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DS</td>
<td>EI %</td>
</tr>
<tr>
<td>( T.) harzianum (Th4)</td>
<td>1.2 b</td>
<td>57.1</td>
</tr>
<tr>
<td>( C.) globosum (Ch2)</td>
<td>1.1bc</td>
<td>60.7</td>
</tr>
<tr>
<td>TH 4+ Ch2</td>
<td>1.0 c</td>
<td>64.3</td>
</tr>
<tr>
<td>TH 4+Sb</td>
<td>1.1bc</td>
<td>60.7</td>
</tr>
<tr>
<td>Ch2+Sb</td>
<td>1.1bc</td>
<td>60.7</td>
</tr>
<tr>
<td>TH4 + Ch2+Sb</td>
<td>0.7 d</td>
<td>75.0</td>
</tr>
<tr>
<td>Ridomil-MZ</td>
<td>0.8 d</td>
<td>71.4</td>
</tr>
<tr>
<td>Control</td>
<td>2.8 a</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Efficiency index (EI) = Control – treatment / Control x 100

Also, many investigators demonstrated that, the potential use of \( C.\) globosum as a biocontrol agent in the management of fungal diseases of potato, citrus, black pepper, strawberry and sugar beet (Tomilova and Shternshis, 2006). Moreover, (Tongon and Soytong 2015) reported that nanoparticles from \( Chaetomium\) globosum successfully control of \( Curvularia\) lunata using leaf spot of rice. Also, \( Trichoderma\) harzianum cultured on sugar cane bagasse, successfully controlled many soil borne pathogens such as \( Fusarium\) solani, \( F.\) oxysporium and \( Macrophomina\) phaseolina, the main pathogens of root rot diseases on grapevines and citrus pepper, celery and citrus (El-Mohamedy, 2004; El-Mohamedy et al., 2010).

Effects on vegetative growth and yield of potato:

Results in Table (4) demonstrated that all applied treatment resulted in considerable increasing in vegetative growth as well as tubers yield of treated potato plants during 2017 and 2018 seasons. The most effective treatments were Th4 Ch 2 grown on 60 g/l (Sb) and fungicides (Redomil – MZ) followed by Th 4+ Ch2 grown on PDA, which resulted in considerable increasing in the plant height, number of leaves/plant and yield kg/plot during two growing seasons as compared with untreated plants. Meanwhile, single treatments of both treatments were less effective. These findings are smaller reported by many investigators. Biological treatments improve the plant growth and reduced disease severity especially with combined treatment this reeect on obtained yield, so the highest records of increasing in potato yield were observed in Th4 + Ch 2 +Sb (72.2 and 67.8%) and Th 4+ Ch2 (62.5 and 58.2%) treatments.
This implies that the de or biological end delays structure filtrate of fast growing
respectively as compared with untreated plants. Effective treatments were co-culture of Th4 + Ch2.
Activities included chitinase and β-glucanase, and enzymes were investigated. Interestingly, Th4 + Ch2 treatments
were significantly effective.

El Gamal et al., Improvement in potential of enzymes

Table (4): Parameters of Potato Plant growth and tuber yield in response to different bio control treatments and Ridomil –MZ during two seasons 2017 and 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season 2017</th>
<th>Season 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. Plant high cm</td>
<td>Av. leaves no.</td>
</tr>
<tr>
<td>Th4</td>
<td>52.0</td>
<td>41.0</td>
</tr>
<tr>
<td>Ch2</td>
<td>50.0</td>
<td>43.2</td>
</tr>
<tr>
<td>Th 4+ Ch2</td>
<td>48.0</td>
<td>40.4</td>
</tr>
<tr>
<td>Th 4+ Sb</td>
<td>52.0</td>
<td>41.2</td>
</tr>
<tr>
<td>Ch2 + Sb</td>
<td>52.0</td>
<td>42.0</td>
</tr>
<tr>
<td>Th4 + Ch 2+Sb</td>
<td>56.0</td>
<td>44.0</td>
</tr>
<tr>
<td>Ridomil MZ</td>
<td>44.0</td>
<td>33.2</td>
</tr>
<tr>
<td>Control</td>
<td>45.0</td>
<td>36.0</td>
</tr>
</tbody>
</table>

The results are on line with finding of (Soytong et al., 2001) they noted that that in greenhouse and field trials, that Tomato, Corn, Rice, Pepper, Citrus, Durian, Birds of paradise and Carnation plants treated with Chaetomium have a greater plant growth and higher yields than non-treated plants. This implies that the mechanism of action for stimulating plant growth is probably confined to our specific strain of Chaetomium. Some Chaetomium species are reported to act as antagonists against various plant pathogens. Even commercial bio-product has also been developed from potent strains of Chaetomium sp. (Soytong et al., 2001).

Effects on chitinase and β-1, 3-glucanase enzymatic activities:
The efficiency of applied biological treatment i.e., T. harzianum (Th4) and C. globosem (Ch2) grown on 60 g/l of soy bean (Sb) or in co cultures on chitinase and β-1, 3-glucanase enzymatic activities in treated and non-treated potato plants was investigated. Results in Table (5) indicate that all treatments of Th4 and Ch2 were significantly increased enzymatic activity in treated plants compared with untreated plants. The most effective treatments were co culture of Th4 + Ch2 grown on agriculture wastes treatments which increased the chitinase (100 and 85.7%) and β-1, 3-Glucanase activity (130.2 and 122.8%) respectively as compared with untreated plants during 2017 and 2018 seasons respectively.

Meanwhile, single treatments showed less effect. In this respect many investigators noted that, T. harzianum and C. globosem induce host defense responses in many plants against many pathogens. There are more than 17 different PR protein families categorized by their properties and functions. Among these PR-1, PR-2, and PR-3 play an important role in antifungal defense, and B-1, 3-glucanase and chitinase contribute to defense response against several pathogens. In this respect, (Song and Soytong 2018) recorded that Chaetomium sp. is commercialized as a new broad spectrum biological fungicide or biological fertilizer. The mechanism of disease control is competition, antibiosis/lysis, antagonism, induced immunity in plants and hyphal interference. It can induce phytoalexin in plants to prevent pathogens and delays onset of disease incidence and delays the development of pathogen resistance. The current study further demonstrated that Chaetomium isolate Cg-6 exhibited higher exo- and endo-glucanase activity in vitro. Similarly, (Hammed et al., 2008) reported higher cellulose activity in the culture filtrate of fast growing Chaetomium isolates. Another interesting observation in the current study was that the Chaetomium isolates produced cellulolytic enzymes which degraded carboxy methyl cellulose (CMC) and cellulose. They also reported

Table (5): Effect of different bio control treatments on β-1, 3-glucanase and chitinase enzymes activities in potato plants and Ridomil –MZ during two seasons 2017 and 2018.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Season 2017</th>
<th>Season 2018</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β-1,3-Glucanase %</td>
<td>Chitinase %</td>
</tr>
<tr>
<td>Th4</td>
<td>6.2</td>
<td>94.0</td>
</tr>
<tr>
<td>Ch2</td>
<td>6.0</td>
<td>88.2</td>
</tr>
<tr>
<td>Th 4+ Ch2</td>
<td>7.0</td>
<td>120.4</td>
</tr>
<tr>
<td>Th 4+ Sb</td>
<td>6.8</td>
<td>112.0</td>
</tr>
<tr>
<td>Ch2 + Sb</td>
<td>6.6</td>
<td>108.2</td>
</tr>
<tr>
<td>Th4+Ch2+Sb</td>
<td>7.4</td>
<td>130.2</td>
</tr>
<tr>
<td>Ridomil MZ</td>
<td>4.8</td>
<td>48.4</td>
</tr>
<tr>
<td>Control</td>
<td>3.2</td>
<td>0.0</td>
</tr>
</tbody>
</table>
that Chaetomium isolates produced extra cellular enzymes which degraded CMC and cellulose. Thus, it is understood from the previous studies that the beneficial microorganisms that secrete greater amount of glucanase enzymes were found to have greater biocontrol activity as the Phytophthora cell wall is predominantly made of glucan and cellulose constituents.

CONCLUSION
Five different fungal isolates of C. globosum and T. harzianum were tested for their antagonistic effect against A. solani, Th4 & Ch2 was the most promising and were used for production of hydrolytic enzymes as free / co-cultured using 60 g/l soy bean (Sb) as carbon source. The co-cultured Th4 & Ch2 grown on Sb were more promising in reduction of early blight of potato, increasing in vegetative growth as well as tubers yield of treated potato plants during 2017 and 2018 seasons. The efficiency of applied biological treatment of Th4 & Ch2 grown on 60 g/l Sb individually or in co cultures on chitinase and β-1, 3-gluconase enzymatic activities increased in treated potato plants.

CONFLICT OF INTEREST
The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS
All authors contributed equally in all parts of this study.

REFERENCES


