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EVALUATION OF RELATIONSHIP BETWEEN CHITINASE PRODUCTION AND BIOCONTROL CAPACITY OF *TRICHODERMA* ISOLATES

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ABSTRACT

Twenty-four fungi were isolated from five plant hosts collected from 15 governorates. The mean percentage of fungal recovery from hosts showed that *Trichoderma* spp. (35.98%) were the most dominant fungi. The other fungi occurred at frequencies ranged from 0.07 to 17.67%. Occurrence of *Trichoderma* spp. was negatively correlated with incidence of five pathogenic fungi. However, the significant correlation was observed between isolation frequency of *Trichoderma* spp. and isolation frequency of *Fusarium* spp. (r= -0.57, p= 0.03). Fifteen isolates of *Trichoderma* spp. were screened for their biocontrol capacity against soil-borne fungal pathogens under greenhouse conditions by using eight pathosystems. These isolates showed various levels of antagonism within each pathosystem. When the same isolates were in-vitro screened for chitinase activity, 20% of the isolates were high producers, 40% were medium producers, and 40% were low producers. Regression analysis was used to study the effect of chitinase activity (independent variable) on percentage of surviving seedlings (dependent variable) in each pathosystem. In most pathosystems, the *in-vitro* efficiency of *Trichoderma* isolates in producing chitinase was not significantly correlated with the percentage of surviving seedlings, which was used as a parameter for evaluating the antagonistic activity of *Trichoderma* isolates under greenhouse conditions. This finding may indicate that the *in-vitro* chitinase activity of *Trichoderma* isolates is of no practical value because it cannot be used as a criterion to predict their *in-vivo* performance. Grouping the isolates by cluster analysis, based on their biocontrol patterns, was not related to their chitinase activity. This result suggests that chitinase may not be involved in the biocontrol process of the tested isolates. Similarly, grouping the isolates by cluster analysis, based on their RAPD banding patterns, was not related to their chitinase activity. This result indicates that RAPD banding patterns were unable to differentiate among the isolates based on their chitinase activity.

INTRODUCTION

Trichoderma Pers. is a genus of Hyphomycetes. Its species are among the most commonly encountered soil fungi (Roiger *et al.,* 1991). *Trichoderma* has been shown to act as a mycoparasite against a range of economically important aerial and soil-borne plant pathogens. Different factors involved in the antagonistic properties of *Trichoderma* have been identified, including antibiotics (Dennis and Webster, 1971 a, b) and hydrolytic enzymes such as β- (1,3) glucanases, proteases, and chitinases (Elad *et al.,* 1984; Geremia *et al.,* 1993). The initial interaction between *Trichoderma* and its host is characterized by the chemotrophic growth of hyphae of the mycoparasite towards the host (Chet and Elad, 1983). When the mycoparasite reaches the host, its hyphae often coil around it or are attached by hook-like structures (Elad *et al.,* 1983a). Following these

interactions, the mycoparasite penetrates the host mycelium, apparently, by partially degrading its cell wall. Susceptible host mycelia show rapid vacuolation, collapse, and disintegration (Elad *et al.,* 1983b; Benhamou and Chet, 1993).

Chitin is one of the main structural components of fungal cell walls (De La Cruz *et al.,* 1992). It is a polymer of N-acetylglucosamine. The enzymatic degradation of chitin by many microorganisms occurs in two consecutive steps. First, chitinase hydrolyzes the polymer to oligomers, mainly dimers, which are subsequently degraded to N-acetylglucosamine by β-N-acetylglucosaminidase (Ueda and Arai, 1992). Although several lytic enzymes may be involved in the complete degradation of mycelial cell walls of phytopathogenic fungi by *Trichoderma,* the data reported provide evidence of major chitinolytic activity and indicate that production of this enzyme is of great significance in antagonistic process (De La Cruz *et al.,* 1992). Therefore, chitinolytic strains of *Trichoderma* are among the most effective biocontrol agents for plant diseases (Agrawal and Kotasthane, 2012).

The main objective of this study was to assess the role of chitinase in the biocontrol activity of *Trichoderma* spp. Prevalence of *Trichoderma* spp. was also evaluated compared with prevalence of other root-colonizing fungi.

MATERIALS AND METHODS

Isolation, identification, and quantification of *Trichoderma* spp. and other fungi from roots of five plant hosts (tomato, cotton, cucumber, peanut, and onion).

Diseased plants at growth stages from seedling to mature plants were collected at random from 15 governorates (Gharbiya, Daqahliya, Minufiya, Ismailiya, Damietta, Sharqiya, Kafr El-Sheikh, Beheira, Qualyubiya, Giza, Fayoum, Beni-Swaif, Minya, Assiut, and Sohag) during 2012 and 2013. Each sample included from 20 to 30 seedlings affected with a variety of damping-off symptoms or rotted roots of 10 to 15 adult plants. The seedlings and roots collected at each field were stored at 4^{o}C until fungal isolation was performed. Seedlings and roots of mature plants were washed thoroughly under running tap water for six hr to remove any adhering soil. Small pieces (approximately 0.5-cm long) of necrotic root tissues were surface sterilized with 10% Clorox solution for 2 minutes, and then washed several times with sterilized water. The surface-sterilized pieces were then blotted dry between sterilized filter papers and plated (5 pieces/plate) onto potato dextrose agar (PDA) medium amended with streptomycin sulfate or penicillin G. and rose Bengal (100-200 mg/L each) as bactericides. The plates were incubated at 26±3^{o}C for 3-7 days. The developing colonies were identified according to Gilman (1966) or Barnett and Hunter (1972). Colonies of each fungus were expressed as percentage of the total developing colonies.

Evaluation of biocontrol capacity of *Trichoderma* isolates by using different pathosystems

Selected isolates of *Trichoderma* spp. were cultured in 500-ml glass bottles, each containing 50 g of sorghum grains and 40 ml of tap water. Bottles with the substrate were autoclaved for 30 minutes prior to inoculation. Isolate inoculum, taken from one-week-old culture on PDA, was aseptically introduced into bottles and allowed to colonize sorghum-grains substrate for 3 weeks. The same method was used for preparing inocula of the pathogenic fungi used in the pathosystems (Table 1). The present study was carried out by using autoclaved clay loam soil. Batches of soil were infested separately with inoculum of each *Trichoderma* isolate at a rate of 50 g/kg of soil. For each *Trichoderma* isolate, eight batches of infested soil (one batch for each psthosystem) were placed on greenhouse benches and separately infested with each of the fungal pathogens (Table 1) at rates of 1, 5, 5, and 1 g/kg of soil for *Rhizoctonia solani*, *Sclerotium rolfsii*, *S. cepivorum*, and *Pythium* sp., respectively. After thoroughly mixing, infested soil was dispensed in 25-cm diameter clay pots and these were planted with 10 seeds of each host or onion transplants (Table 1) per pot. There were five pots (replicates) for each treatment. In the control treatments, *Trichoderma* isolates were not added to the pathogen-infested soil. The greenhouse temperature ranged from 16.5±2.5 to 30.5±3.5^{o}C during the experiment. Percentages of surviving seedlings were recorded 45 days after planting.

Table (1): Pathosystems (Ps) used in evaluating antagonistic activity of *Trichoderma* isolates under greenhouse conditions.

Pathosystem (P)	Host	Pathogen
P_1	Cotton, cv. Giza 89	*Rhizoctonia solani*
P_2	Bean, cv. Bolista	*Rhizoctonia solani*
P_3	Cucumber, cv. Bazindra	*Rhizoctonia solani*
P_4	Sesame, cv. Giza 32	*Rhizoctonia solani*
P_5	Sesame, cv. Giza 32	*Sclerotium rolfsii*
P_6	Sesame, cv. Giza 32	*Pythium* sp.
P_7	Cucumber, cv. Bazindra	*Pythium* sp.
P_8	Onion, cv. Giza 60	*Sclerotium cepivorum*

Screening *Trichoderma* isolates for chitinase activity

Chitinase activity of *Trichoderma* isolates was determined according to the method of El-Katatny *et al*. (2000). In this method, spore-suspension inoculum of each *Trichoderma* isolate (1.0 x 10^6 spores/ml of culture broth) was used to inoculate two 100-ml-flasks containing 20 ml of unbuffered mineral synthetic medium supplemented with dried mycelium of *Sclerotium rolfsii* as the sole carbon source (5 g/l). The cultures were grown at 30^{o}C for 5 days without shaking. Culture filtrates were then centrifuged at 4^{o}C for 10 min at 5000 x g and the clear supernatants were stored at -20^{o}C until assayed.
Chitinase activity was assayed using a colorimetric method. The assay mixture contained 1 ml of 0.5% pure chitin (Sigma, suspended in 50 mM

acetate buffer pH = 5.2) and 1 ml of enzyme solution. The reaction mixture was incubated at 37°C with shaking for 7 hr and was stopped by centrifugation (5000 g/min) for 10 min and the addition of 1 ml of dinitrosalicylate reagent. The amount of reducing sugar released was calculated from a standard curve for N-acetylglucosamine, and the activity of chitinase was expressed in pkat/ml.

Evaluation of genetic diversity of *Trichoderma* isolates by using random amplified polymorphic DNA (RAPD) analysis:

DNA isolation and RAPD analysis

Trichoderma isolates were grown for 22 days at 25-30°C on liquid Czapec medium. The mycelia were harvested by filtration through cheesecloth, washed with distilled water several times. DNA was isolated from 500 mg of fresh mycelium of each isolate using Qiagen kit for DNA extraction (Qiagen, UK). The extracted DNA was dissolved in 100 µl of elution buffer. The concentration and purity of the obtained DNA was determined by using "Gene Quanta" system, Pharmacia Biotech. The purity of the DNA for all samples was between 90-97%. Concentration was adjusted to 6 ng/µl for all samples using TE buffer (pH 8.0).

Polymerase chain reaction (PCR) mixture was prepared with PCR bed tablets (manufactured by Amessham Pharmacia Biotech), which contained all the necessary reagents except the DNA template and the 10-mer primer. The kits of Amessham Pharmacia Biotech also include the following primer: RAPD analysis primer 4:6-d (AAGAGCCCGT)-3.

Thirty ng from each DNA extracted sample and 5 µl of the 10-mer random primer (15 ng/ml) were added to a PCR bead tablets. The total volume was completed to 25 µl using sterile distilled water. The amplification protocol was carried out as follows using PCR unit 11 Biometra: Denaturing at 95°C for 5 min, 45 cycles each consisted of the following steps: Denaturing at 95°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min. Final extension was carried out at 72°C for 5 min and hold at 4°C. Five microliters 6x tracking buffer (manufactured by Qiagen) was added to 25 µl of the amplification product.

Amplification product analysis

The amplified DNA (15 µl) for all samples was electrophoresed using the electrophoretic unit WIDE mini-sub-cell GT (Bio-Rad) on 1% agarose containing 0.5 µg/ml ethidium bromide at 75 constant voltage, and determined with UV transilluminator.

Gel analysis

Gel was scanned for molecular weight (bP) and amount (%) of bands by the gel documentation system AAB (Advanced American Biotechnology, Fullerton CA, 92631, USA). The different molecular weights of bands were determined against DNA standard (G317 A, Promega Inc., USA) with molecular weights 1000, 750, 500, 300, 150, and 50 bp.

Statistical analysis of the data

Analysis of variance (ANOVA) of the data was performed with MSTAT-C statistical package. Least significant difference (LSD) was used to compare

between frequency means of the isolated fungi. Correlation, regression, and cluster analyses were performed with the software package (SPSS 6.0).

RESULTS

Twenty-four fungi were isolated from five plant hosts collected from 15 governorates (Table 2). The mean percentage of fungal recovery from hosts showed that *Trichoderma* spp. (35.98%), *Fusarium* spp. (17.67%), *Rhizoctonia solani* (13.68%), *Pythium* spp. (8.48%), *Sclerotium cepivorum* (5.27%), and *Macrophomina phaseolina* (4.91%) were the dominant fungi. Other fungi occurred at frequencies ranged from 0.07 to 2.46%.

Table (2): Frequency (%) of *Trichoderma* spp. and other fungi isolated from roots of tomato, cotton, cucumber, peanut, and onion.

Fungus	Isolation frequency (%)
Trichoderma spp.	35.98[a]
Rhizoctonia solani	13.68
Fusarium spp.	17.67
Pythium spp.	8.48
Macrophomina phaseolina	4.91
Sclerotium rolfsii	2.46
Sclerotium cepivorum	5.27
Sclerotinia sclerotiorum	0.29
Aspergillus flavus	1.13
Aspergillus niger	0.5
Gliocladium spp.	0.82
Chaetomium sp.	0.53
Botryodiplodia sp.	0.07
Helminthosporium sp.	0.22
Aspergillus spp.	0.37
Cladosporium sp.	0.07
Verticillium sp.	0.07
Nigrospora sp.	0.07
Penicillium spp.	0.29
Alternaria spp.	0.81
Botrytis allii	0.07
Aspergillus nidulans	0.07
Rhizopus nigricans	0.07
Rhizopus sp.	0.07

[a]Mean of five replicates (hosts). LSD (p≤ 0.05) = 8.67

Isolation frequency of *Trichoderma* spp. was negatively correlated with isolation frequencies of five pathogenic fungi (Table 3). The significant correlation was observed between isolation frequency of *Trichoderma* spp. and *Fusarium* spp. ($r = -0.57$, $p = 0.03$).

Table (3): Correlation of isolation frequency (%) of *Trichoderma* spp. with isolation frequency (%) of other fungi from 15 governorates.

Isolation frequency of	Isolation frequency of *Trichoderma* spp.
Rhizoctonia solani	-0.368[a] (0.177)[b]
Fusarium spp.	-0.574* (0.025)
Pythium spp.	-0.480 x (0.070)
Macrophomina phaseolina	-0.423 (0.116)
Sclerotium cepivorum	0.363 (0.184)
Sclerotium rolfsii	-0.016 (0.956)

[a]**Linear correlation coefficient (r) is significant at p<0.05 (*) or p<0.10 (x), and n = 15.**
[b]**Probability level**

Trichoderma isolates showed variable levels of antagonism within each pathosystem (Table 4). For example, isolate T89 was highly effective as a biocontrol agent in pathosystem 2 (P_2) as it increased survival plants by 153.33%. On the other hand, isolate T65 was ineffective. Another example was isolate T84, which increased survival by 100% in P_3, while isolate T102 was ineffective.

Table (4): Evaluation of antagonistic capacity of 15 isolates of *Trichoderma* spp. by using different pathosystems under greenhouse conditions.

Trichoderma isolate (T)	Surviving seedlings (%)							
	Pathosystem (P)							
	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8
T_1	60	75	95	55	25	60	100	50
T_2	60	70	45	80	20	73	93	42
T_3	60	65	85	85	50	73	87	83
T_4	70	70	75	70	20	53	67	58
T_9	60	75	60	60	5	67	100	25
T_{36}	65	50	75	70	25	73	87	100
T_{51}	70	65	40	70	20	43	100	50
T_{65}	30	30	40	50	25	67	100	58
T_{67}	35	60	45	25	15	67	93	25
T_{79}	50	40	70	70	5	67	100	75
T_{84}	50	60	90	90	20	87	80	58
T_{88}	55	60	65	80	35	60	100	58
T_{89}	30	76	60	45	30	60	100	67
T_{102}	25	15	45	40	40	80	93	50
T_{111}	40	50	100	75	15	80	60	67
Control[a]	15	30	45	30	20	53	53	17

[a]**Autoclaved soil was infested only with the pathogen of each pathosystem.**

Fifteen isolates of *Trichoderma* were screened for chitinase activity (Table 5). These isolates could be categorized into three groups: high

chitinase producers (T4, T51, and T89), medium chitinase producers (T1, T2, T3, T9, T36, and T79), and low chitinase producers (T65, T67, T84, T86, T102, and T111). In other words, 20% of the isolates were high producers, 40% were medium producers, and 40% were low producers.

Table (5): Screening of 15 isolates of *Trichoderma* spp. for chitinase activity.

Isolate no.	Chitinase activity (Pkat/ml)	Category
T_1	27.5	Medium
T_2	29.1	Medium
T_3	29.3	Medium
T_4	30.7	High
T_9	27.4	Medium
T_{36}	22.3	Medium
T_{51}	31.3	High
T_{65}	6.5	Low
T_{67}	9.3	Low
T_{79}	27.9	Medium
T_{84}	12.6	Low
T_{88}	14.6	Low
T_{89}	41.7	High
T_{102}	4.4	Low
T_{111}	12.5	Low

Regression analysis was used to study the effect of chitinase activity (independent variable) on percentage of surviving seedlings (dependent variable) in eight pathosystems (Table 6). The two variables were positively correlated in P_1 ($r = 0.53$, $p \leq 0.05$) and P_2 ($r = 0.71$, $p \leq 0.01$), while they were negatively correlated in P_6 ($r = -0.53$, $p \leq 0.05$). The correlation between the two variables was nonsignificant in the other models.

Table (6): Regression equations that describe the effects of chitinase activity (X) by *Trichoderma* isolates on percentage of the surviving seedlings (Y) in different pathosystem.

Pathosystem (P)	Regression equation	F-value[a]	R[b]	R^{2c}
(P_1) *Rhizoctonia solani*- cotton	Y = 2.39 + 0.38 X	4.99*	0.53	0.28
(P_2) *Rhizoctonia solani*- bean	Y = -3.66 + 0.44 X	13.50**	0.71	0.51
(P_3) *Rhizoctonia solani*- cucumber	Y = 17.76 + 0.06 X	0.17	0.11	0.01
(P_4) *Rhizoctonia solani*- sesame	Y = 13.01 + 0.14 X	0.70	0.23	0.05
(P_5) *Rhizoctonia rolfsii*- sesame	Y = 23.46 – 0.07 X	0.08	-0.08	0.01
(P_6) *Pythium* – sesame	Y =56.97 – 0.52 X	5.16*	-0.53	0.28
(P_7) *Pythium* – Cucumber	Y =9.11 + 0.14 X	0.35	0.16	0.03
(P_8) *Sclerotium cepivorum* – onion	Y = 16.14 + 0.10 X	0.42	0.18	0.03

[a]F. value is significant at P≤ 0.01 (**) or P≤0.05 (*).
[b]Linear correlation coefficient.
[c]Coefficient of determination.

A phenogram based on dissimilarity distance (DD) generated from cluster analysis of antagonism patterns of *Trichoderma* isolates is presented in Fig. (1). The smaller the DD, the more closely the isolates were related in their antagonism patterns. Six groups of *Trichoderma* isolates (isolates T84, T111; isolates T3, T79, T36; isolates T2, T9, T51, T88; isolates T1, T4; isolates T65, T102, and isolates T67, T89, respectively) were identified by cluster analysis. Grouping the isolates by cluster analysis was not related to their chitinase activity. For example, isolates T3 and T2 showed almost the same level of chitinase activity: however, they were placed in two remotely related groups. The high chitinase producer T51 and the low producer T88 were placed in the same group. The same observation held true in the case of T87 and T89.

A phenogram based on similarity level (SL) generated from cluster analysis of RAPD banding patterns of *Trichoderma* isolates is presented in Fig. (2). The greater the SL, the more closely the isolates were related in their RAPD banding patterns. Three groups of *Trichoderma* isolates were identified by cluster analysis at SLs 78.60, 82.17, and 75.33%. Isolate T4 showed an unique DNA profile quite different from those of the remaining isolates. Grouping the isolates by cluster analysis was not related to their chitinase activity because isolates of each group showed variable levels of chitinase activity.

Fig.(1): Phenogram based on average linkage cluster analysis of biocontrol capacity of 15 isolates of *Trichoderma* spp. by using eight pathosystems. Biocontrol capacity of the isolates was evaluated based on the surviving seedlings (%). Chitinase activity was expressed as pkat/ml.

Fig. (2): RAPD banding patterns of *Trichoderma* spp. isolates obtained by a random decamer primer and electrophoresed on agarose gel. Chitinase activity was expressed as pkat/ml.

DISCUSSION

The present study demonstrated that *Trichoderma* spp. were the most dominant fungi compared with the other root-colonizing fungi. This result is in agreement with previous reports, which indicated that *Trichoderma* spp. were among the most commonly encountered soil fungi (Roiger *et al.,* 1991).

The isolation of *Trichoderma* spp. from widely separated locations in 15 governorates may suggest that *Trichoderma* spp. are well adapted to colonize plant roots under a wide range of environmental conditions (edaphic factors, crop rotation, irrigation system, temperature and so on).

In the present study, *Trichoderma* spp. were recovered from five plant hosts belonging to five different genera. This may indicate that host specificity in *Trichoderma* spp. was lacking on colonizing plant roots.

The negative correlation between isolation frequency of *Trichoderma* spp. and isolation frequencies of the root-colonizing pathogenic fungi indicates the possibility of antagonism or competition between *Trichoderma* spp. and these soil-borne pathogens. Another possibility is that colorization of plant roots by *Trichoderma* spp. is favoured by environmental conditions, which may not be favourable for colonization of roots by other fungi and vice versa.

Trichoderma isolates showed variable levels of antagonism within each pathosystem. This variability could be attributed to the differential effects of host cultivar and/or pathogen isolate on the performance of *Trichoderma* isolates. That is, a single isolate of a pathogen can be highly sensitive to the application of a single isolate of *Trichoderma* but may exhibit minimal sensitivity to the application of another *Trichoderma* isolate (Asran *et al.,* 2005). Similarly, a host cultivar can be highly responsive to the application of a *Trichoderma* isolate but may show minimal response to the application of another isolate of *Trichoderma* (Asran, 2007).

Production of extracellular chitinase increases significantly when *Trichoderma* spp. are grown in media supplemented with either autoclaved mycelium or isolated purified host fungal cell walls (El-Katatny *et al.,* 2000). These observations, together with the fact that chitin is among the main structural components of most fungal cell walls (De La Cruz *et al*., 1992) are the basis for the suggestion that chitinase produced by some *Trichoderma* spp. play an important role in destruction of phytopathogenic fungi.

Surprisingly, in most pathosystems of the present study, the *in-vitro* efficiency of *Trichoderma* isolates in producing chitinase was not significantly correlated with the percentage of surviving seedlings, which was used as a parameter for evaluating the antagonistic efficiency of *Trichoderma* isolates. This result may indicate that the *in-vitro* chitinase activity of *Trichoderma* isolates is of no practical value because it cannot be used as criterion to predict their *in-vivo* performance under greenhouse conditions.

We suggest some possible explanations for the lack of correlation between the *in-vitro* chitinase production by *Trichoderma* isolates and their *in-vivo* antagonism. It seems reasonable to assume that the biocontrol activity of *Trichoderma* isolates cannot be simply explained by the production of chitinase (Herrera-Estrella and Chet, 1999). This is because the other hydrolytic enzymes and mycotoxins were as important as chitinase or even more important in the biocontrol process of the tested group of *Trichoderma* isolates. Production of chitinase is markedly affected by the pH, with the optimum pH = 6.0 (El-Katatny *et al.,* 2000). The values of pH of the Egyptian soil range from 7.92 to 9.15 (Aly and Kandil, 1999). Therefore, this range is unfavorable for chitinase production by *Trichoderma* isolates applied into the soil. Recognition is an important factor in the mycoparasitic activity of *Trichoderma* isolates (Herrera-Estrella and Chet, 1999). Therefore, the lack of biocontrol activity under greenhouse conditions may be due to the failure of *Trichoderma* isolates to recognize their host (the pathogen).

It is worth noting that the *in-vitro* production of chitinase and the *in-vivo* ntagonism of *Trichoderma* isolates were negatively correlated (r = -0.53,

p≤ 0.05) in the *Pythium*-sesame pathosystem. It is difficult to account for this result and we do not have immediate biological explanation for it.

Grouping the isolates by cluster analysis, based on their biocontrol patterns, was not related to their chitinase activity. This finding is an additional evidence that chitinase may not be involved in the biocontrol process of the tested isolates. Similarly, grouping the isolates by cluster analysis, based on their RAPD banding patterns, was not related to their chitinase activity. This result indicates that RAPD banding patterns were unable to differentiate among the isolates based on their chitinase activity.

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تقييم **العلاقة بين إنتاج إنزيم الكيتينيز والقدرة على المقاومة الحيوية لعزلات فطر التريكوديرما**

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تم عزل 24 فطر من خمسة عوائل نباتية، جمعت من 15 محافظة. أظهرت نتائج العزل أن الفطريات التابعة لجنس التريكوديرما هي الأكثر انتشاراً، حيث وصل تكرار عزلها إلى 35,98%، أما الفطريات الأخرى فقد تراوح تكرار عزلها من 0,07 إلى 17,67 %. وجد ارتباط سالب بين تكرار عزل فطريات التريكوديرما وتكرار عزل خمسة فطريات ممرضة، وكان الارتباط معنوياً في حالة الفطريات التابعة لجنس فيوزاريوم (معامل الارتباط = 0,57 ومستوى المعنوية = 0,03). قيمت 15 عزلة لفطر التريكوديرما، تحت ظروف الصوبة، من حيث القدرة على المقاومة الحيوية، وذلك باستعمال ثماني منظومات مرضية. أظهرت العزلات تباين في القدرة على التضاد داخل كل منظومة مرضية. عندما أختبرت نفس مجموعة العزلات من حيث القدرة على إفراز إنزيم الكيتينيز تحت ظروف المعمل، وجد أن 20% من العزلات كانت ذات قدرة عالية على إفراز الإنزيم و40% ذات قدرة متوسطة و40% ذات قدرة منخفضة. أستعمل تحليل الانحدار لدراسة تأثير إفراز إنزيم الكيتينيز (عامل مستقل) على النسبة المئوية للبادرات السليمة (عامل تابع). وذلك لكل منظومة مرضية. في أغلب المنظومات المرضية موضع الدراسة، كانت كفاءة عزلات التريكوديرما في إفراز إنزيم الكيتينيز تحت ظروف المعمل غير مرتبطة معنوياً بالنسبة المئوية للبادرات السليمة تحت ظروف الصوبة. تدل هذه النتيجة على أن إنتاج إنزيم الكيتينيز بواسطة عزلات التريكوديرما تحت ظروف المعمل ليس له أهمية من الناحية التطبيقية، لعدم القدرة على استخدامه في التنبؤ بأداء هذه العزلات تحت ظروف الصوبة. أستعمل التحليل العنقودي لتقسيم العزلات إلى مجموعات، بناء على ما بينها من تباين في أنماط القدرة على المقاومة الحيوية، إلا أن هذه المجموعات لم ترتبط بقدرة العزلات على إفراز الإنزيم. تدل هذه النتيجة على احتمال عدم مساهمة إنزيم الكيتينيز في عملية المقاومة الحيوية لمجموعة العزلات المختبرة. طبقت تقنية التضاعف العشوائي لمناطق متباينة من الحمض النووي DNA على العزلات، واستعمل التحليل العنقودي لتقسيم العزلات إلى مجموعات بناءً على ما بينها من تماثل في أنماط الحمض النووي، إلا أن المجموعات المتحصل عليها لم ترتبط بقدرة العزلات على إفراز الإنزيم مما يدل على أن تقنية التضاعف العشوائي لا تصلح للتفرقة بين العزلات بناءً على قدرتها على إفراز الإنزيم.