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Role of the Cultural Conditions on the Chitinolytic Activity of *Trichoderma harzianum* on *Sclerotium rolfsii* *Mycelia*

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THE INFLUENCE of environmental and cultural conditions was studied on the chilinolytic activity of *T. harzianum* on both chitin and mycelia of *S. rolfsii* (the causative agent of root rot of a wide range of hosts). The optimum temperature was 30°C, while the best pH-value was 5.0 after 5.0 days of incubation on either homogenized mycelia of *S. rolfsii* or pure chitin. The latter gave more chitinolytic activity in all the treatments. Among the tested carbon sources added beside chitin or the homogenized mycelia, 1.5% molass was the best. 1.0% peptone proved to be the most suitable nitrogen source for maximum chitinases activity. 1.5 and 2.0% of chitin or homogenized mycelia, respectively; gave the maximum values of chitinolytic activity by *T. harzianum*. The cultures of *T. harzianum* were formulated using soya bean flour as a carrier to biocontrol of the root rot disease of *Phaseolus vulgaris*. *T. harzianum* biomass (mycelia resulted after filtration of cultures) was more effective than the whole culture (biomass plus the culture filtrate) in decreasing the percentages of diseased plants compared with control values.

Keywords: *Trichoderma harzidnum, Sclerotium rolfsii,* Root-rot disease, *Phaseolus vulgaris*, Pea-nut, Biocontrol .

Sclerotium rolfsii has a wide host range and causes root-rot and serious damage to soybean, ground nut, beans and other plants (Holcomb, 2000; Thomas & Bhai, 2000 and Garibaldi *et al*., 2000). So, many investigations were conducted for biocontrol of this pathogen with *Trichoderma* species. Kulkarni *et al.* (1994) used *T. harzianum* and *T. viride* to determine their effect on the germination and stand of groundnut seedlings inoculated with *S.rolfsii (Corticium rolfsii),* and they found that *T. harzianum* reduced seedling mortality. Lorito *et al*. (1993) reported that fungal cell wall degrading enzymes produced by the biocontrol fungi *T. harzianum* and *Gliocladium virens)* are strong inhibitors of spore

germination and hyphal elongation of a number of phytopathogenic fungi. Sumitha & Gaikwad (1995) found that *T. harzianum* produced a wide zone of inhibition against *Fusarium u dum* and inhibited spore germination completely. Ghisalberti & Sivasithamparam (1991) reported the mechanisms suggested to be involved in biocontrol by *Trichoderma* species are anti[illegible]ysis, competition, mycoparasitism and promotion of plant growth.

Trichorderma harzianum is known as a mycoparasite of a number of plant pathogens and colonizes *Sclerotium rolfsii* hyphae, disrupts mycelial growth and kills the organism (Ghaffer, 1988). Yan *et al.* (1990) and Xin *et al.* (2000) found that rot disease in pinellas tubers was decreased when tubers were soaked in suspensions of *T. harizanum.* Mycoparasitism is one of the mechanisms by which fungi from the genus *Trichoderma* antagonize other fungi and serve as a bicontrol agents of plant diseases (Chet, 1990; Mishra *et al.*, 2000 and Sarhan *et al.*, 1999). Lea *et al.* (1997) reported that mycoparasitism seems to be the main mechanism in control *S. rolfsii.* Ansari & Agnihorti (2000) and Hermosa *et al.* (2000) reported that different *Trichoderma harzianum* strains differ in its ability to antagonize phytopathogens due to variation in toxic substances and lytic enzymes production as well as growth rates. Also, Lea *et al.* (1997) and Biswas *et al.* (2000) attributed the mycoparasitism of *S. rolfsii* by *T. harzianum* to lytic enzymes production.

However, Wilhite & Straney (1996) found that *Gliocladium virens (Trichoderma virens)* antagonize the pathogens by production of epithiokitopiperazine antibiotic called gliotoxin. Wilhite *et al.* (1994) stated that *Gliocladium virens* strain G2o (syn GL 21) has been commercially formulated into the disease suppressing product Gliogard TM.

Material and Methods

Fungal isolates

Sclerotium rolfsii and *Trichoderma harizanum* isolates were obtained from Department of Seed Pathology, Plant Pathology Institute, Agriculture Research Centre, Giza, Egypt.

Pathogenicity test

It was carried out under the greenhouse conditions in pots (30 cm diameter). Pots were sterilized by immersion in 50% formalin solution for 5 min and left for several days. The used sandy loam soil was sterilized by autoclaving at 121°C for one hour three separate times. Sclerotia of *S. rolfsii* were used for infesting of the soil. Five healthy kidney bean seeds *(Phaseolus vulgaris)* were sown in each pot. The pathogen was then isolated from the diseased plant and used again to infest healthy plants.

Culture medium

Richard's medium (Riker & Riker, 1936) was used. Its constituents was as follows (g/L): Sucrose, 50.0; sodium nitrate, 10.0; potassium dihydrogen phosphate, 5.0., magnesium sulphate. Pentahydrate, 2.5. and distilled water up to 1000 ml.

Effect of different culture conditions

The effect of incubation temperature, incubation periods and pH-values as well as different carbon and nitrogen sources were studied using a basal medium containing 1% colloidal chitin or 1% homogenized *S. rolfsii* mycelia instead of the carbon source of the basal medium.

Estimation of the chitinolytic activity

The cultures of *Trichoderma harzianum* were centrifuged at 5000 rpm for 15 min in refrigerated centrifuge. The clear supernatant was considered as the source of the crude chitinase enzyme. Colloidal chitin was prepared as described by Jeuniaux (1966). Commercial chitin (Sigma Chemical Company) was successively treated with cold IN HCl, 0.5N NaOH at 100ml, 0.5% $KMnO_4$ at 60°C (20 minutes), cold half saturated $Na_2S_2O_3$, hot water and then ethyl alcohol. The purified chitin was then ground in distilled water (waring blender). The obtained pellet was compeleted to a known volume with distilled water. A known volume was then taken and dried in an electric oven (100°C) till dryness to know the actual dry weight. The dried colloidal chitin was suspended in acetate buffer (pH 5.2) (Jeuniaux, 1966). The chitinolytic activity was measured according to Monreal & Reese (1969). In which, the amount of the liberated N-acetyl glucosamine of the assay mixture was then determined using the colourimetric determination method described by Reissig *et al.* (1955). Chitinase unit was determined according to the definition of Monreal & Reese (1969) as the amount of enzyme in 1 ml required to produce 1.0 mole of N-acetyl glucosamine in one hour at 37°C. Specific activity of the enzyme was achieved to determine the number of units per mg protein.

Protein amount

It was estimated by folin phenol reagent according to the method of Lowry *et al.* (1951). To avoid reaction of folin reagent interference with the components of the culture filtrate, precipitation was carried using acetone at 5°C for 24 hr. The samples were centrifuged at 5000 rpm for 15 min in a refrigerated centrifuge. After which, the precipitate was suspended in 5 ml of alkaline solution. Folin reagent was then added and the developed colour was measured colourmetrically at 700 nm. Standard curve was made using egg albumin (20-200 micrograms/ml).

Application and delivery of the biocontrol agens

Trichorderma harzianum biomass or cultures were used for biocontrol of *S. rolfsii* the causal agent of the root-rot of kidney bean plant. Cultures were

obtained after growth of *T. harzianum* on the best conditions using the basal medium supplemented with 1% homogenized *S.rolfsii* mycelia as a carbon source. *T. harziamum* biomass or cultures were mixed with soya bean flour as a food base carrier (1 : 1 W/W) and applied as seed-dressing at a rate of 10g/kg seeds. Six replicates pots were grown in presence of biocontrol agent and the pathogen or in presence of the biocontrol agent alone or in presence of the pathogen alone or in absence of the both. After which, the percentages of diseased plants were determined.

Results and Discussion

Considerable attention has been focused on the isolation of fungal antagonists that could be as effective as a pesticide in repressing fungal pathogens (Benhamou and Chet, 1996). Chitinolytic enzymes are known to perform several biological functions. They are required for the morphogenesis of fungi (Cabib *et al.*, 1992). They may play a nutritional role for soil saprophyte microorganisms (Monreal & Reese, 1969). Plant chitinases are involved in defence reactions against pathogens, either through a direct inhibitory effect (cell wall lysis) or via an indirect effect (release of fungal cell wall elicitors) (Roberts & Selitemnikof, 1988). *Trichoderma harziamum* was known as a mycoparasite of a number of plant pathogens (Ghaffer, 1988). Lea *et al.* (1997) and Biswas *et al.*, (2000) attributed the mycoparasitism of *S. rolfsii* by *T. harzianum* to lytic enzymes production. *Trichoderma* spp are known to be efficient producers of polysaccharide lyase, proteases and lipases, all of which may be used for degradation of cell wall of target fungi (Cherif & Benhamou, 1 990 and Harman *et al.*, 1993). Lorito *et al.* (1994) reported that different classes of cell wall degrading enzymes produced by the biocontrol fungi *Trichoderma harzianum* and *Gliocladium virens* inhibited spore germination of *Botrytis cinerea* in a bioassay *in vitro*. Lorito *et al.* (1994 a) found that four cell wall degrading enzymes were tested as mixtures containing two, three or all four proteins in all possible combinations. A synergistic, inhibitory effect was detected on both spore germination and germ tube elongation of *Botrytis cinerea*. The highest level of inhibition was obtained when a solution containing four cell wall degrading enzymes was used. The enzymes include a glucan 1,3-B-glucosidase and an N-acetyl-B- glucosaminidase. They were isolated and purified from *Trichoderma harzianum* P 1. Other enzymes are produced by *Trichoderma* spp. such as xylanase (Toerroenen *et al.*, 1994) and cellulase (Thrane *et al.*, 1997). The main lytic enzymes are chitinases enzymes produced by *Trichoderma* species. So, this work was localized upon studying of the optimum factors needed by *Trichoderma harzianum* to produce maximum amounts of chitinases enzymes on homogenized mycelia of *Sclerotium rolfsii* to be used in biocontrol of the latter pathogen.

It is evident from Table 1 that *T. harzianum* was able to grow at all the tested incubation temperatures. The optimum incubation temperature for chitinase production was 30°C on chitin or homogenized mycelia. Lower or higher temperatures yielded chitinases with lower productivity. Kapat *et al.* (1996) found that the optimum incubation temperature for maximal chitinase productivity by *T. harzianum* was 30°C. The same temperature was found to be the optimum one by Ulhoa & Peberdy (1991); Sabry *et al.* (1992) and Khalaf (1993).

TABLE 1. Average values of chitinase activity (units/ml) protein (mg/100ml) and specific activity (units / mg protein) of *Trichoderma harzianum* incubated at different incubation temperatures (°C).

Temperature (°C)	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
15	0.2	0.0	6	50	3.33	0.00
20	8.1	1.5	33	31	24.54	4.83
25	12.5	10.5	40	37	31.25	26.25
30	18.2	13.3	49	47	37.1	28.27
35	11.5	9.5	35	30	32.4	31.66
40	5.5	3.3	30	28	18.33	11.78
45	1.5	0.5	15	12	10.0	4.16

It is clear from Table 2 that pH 5 was the optimum pH value for maximum chitinolytic activity by *T. harzianum*. Protein and specific activity values were increased with pH values increasing reached their maximal values at pH 5.0. El-Sherbeiny (1990) found that the acidic side was preferable for *Trichoderma* spp. to obtain maximum antagonistic potential against the tested phytopathogenic fungi. The maximum value of chitinolytic activity of *Trichoderma harzianum* was obtained after 5 days (Table 3). The values of chitinases were decreased by extending of the incubation period. Sarhan *et al.* (1999) found that *T. hamatum* released chitinase enzyme when grown in dual culture with *Fusarium oxysporum* f. sp. *lycopersici*. They reported that the optimum incubation period was 4 days.

Nutritional requirements are very important for chitinase production by chitinolytic microorganisms. With respect to the influence of addition of different carbon sources to chitin or mycelia basal salt medium. The results in Tables 4

TABLE 2. Average values of chitinase activity (units/ml) protein (mg/ml) and specific activity (units/mg protein) of *Trichoderma harzianum* cultivated on different pH-values adjusted with citrate buffer:

PH-values	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
2.6	4.0	3.0	12.9	9.0	31.0	33.33
3.0	5.5	3.5	15.7	10.0	35.0	35.0
4.0	14.0	12.0	38.8	33.0	36.1	36.36
5.0	18.2	16.0	49.0	42.0	37.14	38.09
6.0	13.1	12.0	43.0	40.0	30.46	30.0
7.0	7.1	5.0	43.0	41.0	16.5	12.19
8.0	3.1	2.0	30.0	25.0	10.33	8.0

and 5 indicate that molass, as a carbon source, in addition to chitin or mycelia, was suitable to give the maximum amount of chitinase by *Trichoderma harzianum* Mohamed (1989) found that sucrose was the best carbon source for growth of *Trichoderma lignorum.* Biosynthesis of chitinase in filamentous fungi as in most microorganisms is thought to be controlled by induction-repression mechanism, *i.e.* chitin or products of chitin degradation regulate the chitinase activity. This finding was explained by the repressor-induction system as described by Vasseur *et al.* (1990). Starch induced the production of an amount of chitinase lesser than control (chitin or mycelia only). On the other hand, the induction of chitinase productivity by insoluble chitin could result from the physical contact between the cell surface and the insoluble chitin. This fact was indicated by Ulhoa and Peberdy (1991) and Mohamedin (1993). The latter used *Streptomyces reticuli* in his study. Mahadevan and Craw Ford (1997) indicated that addition of polysaccharides with chitin to the culture media increased chitinase production, and these results were in harmony with the results of the present work in case of addition of carboxymethyl cellulose and not in case of starch addition.

TABLE 3. Effect of different incubation periods on chitinase activity (units/ml), protein (mg/100ml) and specific activity (units/mg protein) of *Trichoderma harzianum*.

Incubation periods (days)	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
1	0.0	0.0	5	1	0.0	0.0
2	1.0	0.9	9.1	8	10.989	11.25
3	8.0	7.3	25	22	32.0	33.18
4	13.1	12.2	39	35	33.589	34.857
5	18.2	16.3	49	43	37.142	37.906
6	17.1	15.5	47	43	36.382	36.046
7	15.5	13.2	49	42	31.632	31.428
8	15.5	13.0	48	40	32.291	32.50
9	13.9	12.4	45	38	30.888	32.60
10	11.1	11.0	40	37	27.25	29.72
11	10.5	10.4	38	37	27.63	28.108
12	10.3	10.1	38	36	27.105	28.055
13	9.5	9.0	36	35	26.388	25.714
14	9.0	8.1	35	33	25.714	24.54

Chitinase production by *T. harzianum* was not only affected by the type of carbon source used but also was sensitive to the concentration of the specific carbon source present in the growth medium. As apparent from Table 5, 1.5% molass was the optimum concentration for production of the maximum value of chitinase and any increase in the amounts of molass concentration was accompanied with a clear increase in the amounts of chitinase activity, protein and specific activity. The yield of chitinase depends on the organism and the nature of the sugar added with the chitin (Sabry *et al*., 1992).

TABLE 4. Average values of chitinase activity (units/ml) protein (mg/100ml) and specific activity (units/ mg protein) of *Trichoderma harzianum* cultivated on different carbon sources.

Carbon sources	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
Control	18.2	16.8	49	48	37.1	35.0
Glucose	19.0	16.9	80	60	23.8	28.2
Galactose	18.5	16.0	71	66	26.1	24.2
Sucrose	20.0	17.5	75	70	26.7	25.0
Carboxymethyl cellulose	22.0	19.0	60	55	36.7	34.2
Starch	18.0	15.5	50	44	36.0	35.2
Fructose	20.0	17.0	70	66	28.6	25.8
Lactose	26.0	20.0	67	60	38.8	33.3
Maltose	18.9	17.1	52	43	36.3	39.8
Molass	30.0	2.3	80	71	37.5	32.3
Venasse	28.0	21.0	78	68	35.9	30.9

Table 6 indicates that chitinase activity of *T. harzianium* was also affected by the nature and type of the nitrogen source. All the tested nitrogen sources exhibited a clear increase in the values of chitinase, protein and chitinase specific activity. Maximum amounts of chitinases of *T.harzianum* was produced on peptone nitrogen source by other workers as Elad *et al.* (1982). They found that ammonium nitrate induced the highest chitinase production by *T. harzianum* Papavizas (1985) reported that ammonium salts were the most readily utilized nitrogen by *Trichoderma* spp. El-Naghy *et al.* (1985) indicated that chitin itself

could be used as a carbon and nitrogen source for growth of *A. fumigatus, T. viride, Stachybotrys atra, T. harzianum* and *Mucor racemosus.* Table 7 indicates also that varying concentration of peptone excreted a marked effect on chitinase production by the test organism. Chitinase production was increased with the increase in nitrogen level and reached its maximum value at 1.0% of peptone, any concentration above or below that optimum level gave relatively inferior chitinase production by the selected fungus. Differennt authors reported that chitinase production is influenced by the nitrogen concentration in the media (Elad *et al.*, 1982; Sherief *et al.*, 1991; Sabry *et al.*, 1992 and others).

TABLE 5. The values of chitinase activity (units/ml) protein (mg/100ml) and specific activity (units/mg protein) produced by *Trichoderma harzianum* cultivated on different concentrations of molass.

Molass Conc (%)	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
Control	18.2	16.8	49	48	37.1	35.0
1.0	30.0	23.0	80	71	37.5	32.2
1.5	40.1	25.0	85	70	47.2	43.3
2.0	38.0	23.3	82	68	46.3	32.4
2.5	30.5	20.1	62	62	43.6	32.4
3.0	22.5	19.2	61	61	36.9	31.5

As apparent from Table 8, it is evident that 1.5% chitin (w/v) or 2% homogenized mycelia of *S. rolfsii* were the best for maximum amounts of chitinase activity, protein and chitinase specific activity of *T. harzianum.* This fungus released chitinase enzyme in dual cultures with *Sclerotium rolfsii* (Elad *et al.*, 1984) or with *Lentinus edodes* (Tokimota, 1982) and this confirms the results of the present paper. Production of chitinases was reported to be controlled by a repressor– inducer system in which chitin or its products of degradation (oligmers) serve as an inducer (Ulhoa & Peterdy, 1991 and Zheng *et al.*, 1994). Abdel-Fatah (1995) found that 1.5% Chitin was the best concentration for chitinase production by *Streptomyces cellulosae,* while Mahadevan & Crawford (1997) found that 1% chitin was the best concentration for *Streptomyces lydicus.*

TABLE 6. Average values of chitinase activity (units/ml) protein (mg/100ml) and specific activity (units/mg protein) of the experimental organism cultivated on different nitrogen sources:

Nitrogen sources	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
Control	9.5	8.0	33	30	28.8	26.7
Ammonium phosphate	16.1	15.5	46	44	35.0	35.2
Ammonium sulphate	20.1	16.0	50	42	40.6	38.1
Ammonium chloride	19.5	16.1	41	35	47.2	46.0
Glycine	25.5	24.0	54	51	47.2	47.0
Potassium nitrate	18.2	16.8	49	48	37.1	35.0
Peptone	26.2	25.0	60	58	48.3	43.1
Sodium nitrate	23.2	20.2	55	51	42.1	39.6
Urea	10.1	9.0	35	33	28.9	27.3

An innovative approach in the formulation of microbial pest control agents, which has captured the interest of scientists as well as of industry, involves the immobilization or entrapment of biomass within a cross-linked matrix of organic polymers such as alginate, polyacrylamide or carrageenan (Wood, 1985). Experimental powder formulation of fungal biological control agents such as *Trichoderma viride* and *Gloicladium virens* had been successfully prepared using pyrophyllite as a carrier substancep (Papavizas & Lewis, 1989). Embedding propagules of biocontrol agents in the matrix of sodium alginate and bulking agents such as clay, lignite or bran has been shown to be an effective way of delivering most fungal biocontrol agents, particularly *Gliocladium spp* and *Trichoderma* spp for the control of root rot and damping off diseases (Lewis & Papavizas, 1987).

TABLE 7. Effect of different nitrogen levels on the production of chitinase activity, protein content (mg/100ml) and chitinase specific activity (units/mg protein) of by *Trichoderma harzianum*.

Nitrogen level	Chitinase activity (units/ml)		Protein (mg/100 ml)		Chitinase activity (units/mg protein)	
	Chitin	Mycelia	Chitin	Mycelia	Chitin	Mycelia
Control	9.5	8.0	33.0	30.0	28.8	26.7
1.0	26.0	20.0	67.0	60.0	38.8	33.3
1.5	22.5	19.2	61.0	61.0	36.9	31.5
2.0	20.6	17.5	75.0	70.0	26.7	25.0
2.5	13.1	12.0	43.0	40.0	30.46	30.0
3.0	10.1	19.0	35.0	33.0	28.9	27.3

TABLE 8. Average values of chitinase (units/ml), protein (mg/100ml) and specific activity (units/mg) of the experimental organism cultivated on different concentrations of chitin or homogenized *S. rolfsii* mycelia.

(%) of chitin or mycelia	Chitin			Mycelia		
	Chitinase activity (u/ml)	Protein mg/ 100 ml)	Specific activity (u/mg protein)	Chitinase activity (u/ml)	Protein mg/ 100 ml)	Specific activity (u/mg protein)
0.5	9.0	30	30.0	6.0	20	30.0
1.0	14.8	40	37.0	12.0	33	36.4
1.5	18.2	44	37.1	14.8	40	37.4
2.0	13.2	41	32.4	16.8	48	35.0
2.5	13.0	42	30.95	11.0	44	32.7
3.0	10.0	33	30.30	11.0	36	30.6
3.5	6.5	24	27.08	10.0	33	30.3
4.0	5.0	19	26.31	6.0	25	24.0
4.5	4.2	17	24.70	3.0	13	23.1

TABLE 9. Effect of application of *Trichoderma harzianum* to the infested soil on the disease incidence in *Phaseolus vulgaris*.

Treatments	Delivery form	% of diseased plants		% of healthy plants (healthy survival)
		Pre- emergence	Post-emergence	
Control (Pathogen alone)	-	86.0	14.0	0.0
T. harzianum	Soybean flour: biomass (1:1)	30.0	6.0	64.0
	Soybean flour: whole culture suspension (1:1)	34.0	7.0	59.0

The application of biocontrol agents to seeds, seed pieces or tubers appears to represent the most suitable method (Lewis, 1990). Greenhouse experiment of this work indicated that application of *T. harzianum* biomass or culture as two delivery forms using soybean meal as a carrier (Table 9) reduced the percentages of both pre-emergence and post emergence diseased plants compared with the control treatment. The effect of using biomass of *T. harzianum* was better than using the whole culture. *T. harzianum* has another advantage beside the production of chitinases. This is the production of antifungal material which inhibitis the germination of *S. rolfsii* sclerotia and its mycelial growth (Reddy *et al.*, 1992). The use of wet biomass or whole suspension was advantageous because drying and milling steps eliminate the effcet of the biocontrol agents (Papavizas & Lewis, 1989).

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تأثير الظروف المزرعية على إنتاج إنزيم الكيتيناز بواسطة فطرة التريكودرماهارزنيم على هيفات فطرة اسكلروشيوم رولفيساى

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إستخدمت الهيفات المطحونة لفطرة أسكلروشيوم رولفيساى كمصدر للكيتين وذلك لدراسة تأثير العوامل المزرعية على إنتاج إنزيم الكيتيناز بواسطة فطرة التريكورماهارزيـانيم. إتضح أن درجة الحرارة المثلى كانت ٣٠°م بينما كان أفضل رقم هيدروجينى للوسـط الغذائى هو خمســة، وكانــت فتــرة التحضــين المثلــى هــى خمســة أيــام لفطــرة التريكودرماهارزنيم على الكيتين أو الهيفات المطحونة لفطرة اسكلروشيوم رولفيســاى، علماً بأن الكيتين كان أفضل من الهيفات المطحونة من حيث إنتاج إنزيم الكيتيناز بواسطة فطرة التريكودرما هارزيانيم. وإتضح أن ١,٥٪ مولاس هو أفضل مصدر كربونى وأن ١٪ بيتون هو أفضل مصدر نيتروجينى لإعطاء أقصى كمية من إنزيم الكيتيناز مقارنــة بالمصادر الكربونية والنيتروجينية الأخرى. وإتضح أن أحسن تركيز مـــن الكيتـــين أو الهيفات المطحونة كان ١,٥، ٢٪ على الترتيب. تـــم إســـتخدام مزرعـــة التريكودرمـــا هارزيانيم كاملة أو إستخدام الكتلة الحيوية فقط للمقاومة الحيوية لمرض عفـــن الجـــذر لنبات الفاصوليا مخلوطه بنسبة ١:١ مع دقيق فول الصويا، وإتضح أن الكتلـــة الحيويـــة لفطرة التريكودرما هارزيانيم كاملة هى الأفضل.