BIODEGRADATION OF WASTEPAPER BY *TRICHODERMA VIRIDE* AND USING THE BIOPROCESSED MATERIALS IN BIOCONTROL OF DAMPING-OFF OF PEA CAUSED BY *PYTHIUM DEBARYANUM*

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ABSTRACT

The biodegradation of wastepaper materials such as filter paper, foolscap, cardboard, tissue paper and newspapers was investigated. By using a method based on clear zone formation on agar plates, a total of 100 microbial strains (comprising fungi and bacteria) could be isolated from different microbial sources using carboxymethyl cellulose as substrate for cellulase. Sixteen cellulylotic microorganisms from the 100 isolated (comprising fungi and bacteria) as potential cellulase producers were selected. One fungal strain of 16 cellulylotic microorganisms exhibited wider clear zone than the other strains. This strain was identified as *Trichoderma viride*. The optimal pH and temperature for growth *T. viride* and its cellulase production were 6.5 and 25°C, respectively.

It was shown that the cellulase was induced in submerged culture with presence of the carboxymethyl cellulose and wastepaper materials in MSL, while the presence of additional carbon sources such as glucose or a complex media (Potato Dextrose) suppressed enzyme production.

All wastepaper materials exhibited different susceptibilities towards cellulase to their conversion to reducing sugars. The present study showed also that, the general trend of bioconversion of different wastepaper materials with cellulase was more than the general trend of bioconversion of different wastepaper materials by *T. viride*.

Additionally to the qualitative clear zone tests, the degradation potential of *T. viride* was characterized via weight loss measurements of wastepaper materials on agar plates. *T. viride* exhibited also good degradability for mixture from the tested wastepaper materials in solid state fermentation without newspapers leads to produce biomass (bioprocessed materials).

Results of this study suggest that soil treatment with the bioprocessed materials was an effective in controlling damping-off of pea and could be considered as promising alternative to existing

chemical products, where the effects were similar in more cases to those of maxim fungicide. The application of these results may be help in reducing the effect of environmental pollution which caused by wastepaper materials or chemical fungicides.

Keywords: Biodegradation. *Trichoderma viride*, cellulase, wastepaper. *Pythium debarayanum*, soil treatment, biological control

INTRODUCTION

Various wastepaper materials are a major component of solid waste all over the world. Cellulolytic enzymes play an important role in natural biodegradation process in which plant lignocellulosic materials are efficiently degraded by cellulolytic fungi and bacteria. In industry, these enzymes have found novel applications in the production of fermentable sugars and ethanol (Olson and Hahn-Hagerdahl, 1997; Levy *et al.*, 2002; Van Wyk and Mohulatsi, 2003), organic acids (Luo *et al.*, 1997), detergents, and other chemicals (Oksanan and Peeabilaniana 1998). They have been used in the pulp and paper industry, e. g., in deinking of fiber surfaces and in improving pulp drainage (Oksanan *et al.*, 2000; Suurnakki *et al.*, (2004)), in the textile industry (Cavaco-Paulo and Gübitz (2003), Nierstrasz, and Warmoeskerken, 2003; Miettinen-Oinonen *et al.*, 2004), animal feed (Ishikuro, 2000), and even in the food industry(Penttila *et al.*, (2004); Urlaub, (2002), for the processing of paper and cellophane, as well as for biotransformation of wastepaper to fermentable sugars (Van Wyk and Mohulatsi, 2003). As lytic enzymes, they are of prime importance is the protoplast production (Davis, 1985; Mandels, 1974; Bhat, 2000). Fungal cellulases are inducible enzymes that are usually excreted into the environment (Bhat and Bhat, 1997) and depend on cellulose type (amorphous or crystalline) acting on the organism (Ortega *et al.*, 2001). The role of the fungi *Acremonium* spp., *Chaetomium* spp., *Trichoderma reesei*, *Trichoderma viride*, *Penicillium pinophilum*, *Phanerochaete chrysosporium* (*Sporotrichum pulverulentum*), *Fusarium solani*, *Talaromyces emersonii*, *Trichoderma koningii*, *Fusarium oxysporum*, *Aspegillus niger* and *Rhizopus oryzae* in the cellulose degradation process in various environments has been well documented (Kuzmanova *et al.*, (1991); Teerei and Koivala, (1995); Bhat and Bhat. (1997); Schülein, 1997; Murashima *et al.*, 2002; and Mach, Zeilinger, 2003).

Many investigators reported earlier the use of *Trichoderma hamatum* or *T. harzianum* for the control of *Pythium* seed rot and

Rhizoctonia root rot in different crops such as pea (Harman *et al.*, 1980, Nelson *et al.*, 1988 and Belal *et al.*, 1996).

Therefore, the present investigation is an attempt to study the biodegradation of wastepaper, bioconversion of the tested wastepaper to fermentable sugars as well as using the bioprocessed materials in controlling of damping –off of pea caused by *Pythium debaraynum*.

MATERIALS AND METHODS

Media

Minimal Medium as mineral salt medium (MSL) was used through this study as described by (Drews 1968) and Luria Bertani Medium (LB), TSB as well as Potato Dextrose Agar (PDA) were used also as complex media in the present study.

Sampling and Cellulolytic microorganisms isolation

Samples of the different substrates–raw materials (wastepaper), mushroom fruits, soil, mature compost, primary effluent mud, wastewater from the sedimentation reservoir were collected from the paper factories located in different Governorates in Egypt.

In laboratory, 1 g of each substrate (some of which needed to be milled) was added to the conical flask containing 99ml of MSL medium and mixed for 30 min on a rotary shaker (150 rpm) at room temperature. Samples of sludge and wastewater were added as 1 ml to 99 ml of MSL medium. The supernatants were separated by centrifugation at 5000rpm for 20min. Ten-fold dilutions were prepared and then 100µl of each dilution were spread on plates containing MSA + carboxymethyl cellulose (10 g/L as a sole source of carbon) using drigalisky triangle. The plates were sealed in polyethylene bags and were incubated at 25°C for 7days monitored for appearance of colonies. Cellulolytic strains were selected on the basis of the diameter of the hydrolysis zone surrounding the colonies as described by (Teather, Wood, 1982; Bradner *et al.*, 1999 and Peciulyte 2007). Single colonies growing on the dilution plates were isolated by picking the colonies using sterile inoculation needle and were further purified by the standard spatial streaking for bacterial isolates on complex agar media (TSB or nutrient agar) and or using acidic complex medium or addition of ampicilline 800mg/L to complex medium for fungal isolates (PDA for fungal isolates).

Identification

The selected wastepaper degrading bacterial strains were identified as described by Bergy's manual of systematic bacteriology (1984). Also, the selected wastepaper degrading fungal strains were

identified according to Rifai, M. A. (1969), Domsch *et al.*. 1980, and Burgess *et al.*, (1994).

Degradation of different wastepapers materials by the microorganisms via measuring of clear zone

The isolated colonies were then tested for their ability to grow and degrade different wastepaper materials (foolscap, filter paper (whatman paper No.1), cardboard (packing materials), tissue paper, newspaper) in MSL medium. All wastepaper were milled. 100ml MSL medium containing 10g/L from each material was inoculated by 3 ml from fungal suspension at 10^6 cfu/ml or bacterial cell suspension at 10^7 cfu/ml, respectively. One treatment contained the medium and carboxymethyl cellulose and the other contained the medium without carboxymethyl cellulose and the isolate (control).

The cultures were shaken at 150rpm and 25°C for 14 days. All assays were carried out from cultures supernatant as extracellular cellulase source after removing the growth by using sterile membrane filter (0.2µm). 50µl of culture supernatant was added in wells (5mm in diameter) of MSA (containing carboxymethyl cellulose 10 g/L as substrate). The plates were treated and the clear zone was measured according to the method described by Teather, Wood, (1982); Bradner *et al.*. (1999) and Peciulyte (2007).

Effect of pH and temperature on growth of *Trichoderma viride* **and its cellulase (CMCase) production**

One hundred ml MS-medium supplemented with carboxymethyl cellulose (10 g/L) as a sole source of carbon were used to determine the effect of pH and temperature on growth of *T. viride* and its cellulase production. The medium was inoculated by 3ml (10^6 cfu/ml) of culture of *Trichoderma viride* strain. The experiments were carried out at pH 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5 and 8 and the culture was incubated at 25°C with shaking (150 rpm) for 14 days. To determine the optimum temperature, MSL medium at pH6.5 was incubated at 20, 25, 30, 35 and 40°C with shaking (150 rpm) for 14 days. The activity of *Trichoderma viride* cellulase was determined by measuring of clear zone as described above. The growth was determined as mycelial dry weight of biomass (g) after 14 days as described by Belal (2003).

Effect of different carbon source on growth *Trichoderma viride* **and enzyme induction**

One hundred ml MSL in conical flasks (250ml) containing 10g/L (all wastepaper materials were milled) from each material or glucose was inoculated by 3 ml from fungal suspension at 10^6 cfu/ml

(one-week-old colonies of fungi grown at 25 °C on PDA plates). Potato dextrose (PD) was used as complex medium and it was carried at the same conditions. Cultures were incubated in shaker incubator for 14 days at 25°C and 150 rpm. After 14 days of cultivation, culture aliquots were centrifuged at 5000 rpm to remove solids. The supernatants were assayed for their enzymatic activity by measuring of clear zone as described above. The growth was determined as mycelial dry weight of biomass (g) after 14 days as described by Belal (2003).

Enzyme assay and saccharification of wastepapers materials by the *Trichoderma viride* cellulase

Cellulase activity was determined by incubating 0.5 ml of the supernatant (at a concentration of 250 µg/ml, while the enzyme concentration was determined according to Lowry *et al.*, 1951) with 0.5 ml of an amount 10g/L of each material carboxymethyl cellulose or milled wastepaper materials in 0.05 M citrate buffer (pH4.8) at 50°C for 30min. After incubation, the reaction was terminated by adding 3 ml of 1% 3,5-dinitrosalicylic acid (DNS) reagent to 1 ml of the reaction mixture and heated for 10min. In these tests, reducing sugars were estimated calorimetrically after Miller (1959), using glucose as standards. One unit of cellulase activity is defined as the amount of enzyme that releases 1 µmol reducing sugars (measured as glucose) per ml per min.

Biodegradation of different wastepaper materials by *Trichoderma viride*

Weight loss determination of wastepaper films on agar plates

The degradation test was carried out with wastepaper films (30 – 60mg) on MSA as a sole source of carbon at the optimal growth conditions. Three preweighted, sterile circular films (25 mm diameter, surface area assessable for degradation (4.91 cm^2) of the wastepaper were placed on a MSA- plate and inoculated with 80µl from fungal suspension at 10^6 cfu/ml on surface of the wastepaper film. The degradation times on the agar plates was 3 weeks at the optimal growth conditions. Sterile controls incubated over the same period of time were performed and showed no weight loss due to a biotic hydrolysis of the paper samples according to the described methods of Belal (2003) with replacing plastic films with wastepaper films.

Biodegradation of mixture of wastepaper materials by *Trichoderma viride* by using solid state fermentation and using the produced biomass (bioprocessed materials) in suppression of *Pythium debarayanum*

Different wastepaper materials (filter paper(whatman paper No.1), foolscap, , cardboard (packing materials), and tissue paper were prepared in pieces of 3cm × 3cm and mixed well with ratio of 1:1:1:1 and placed in glass box (width 40cm × 30cm height). The mixed wastepapers were moistened (till 65%) with MSL medium without addition any carbon source, the substrate moistened when needed. The mixed wastepaper materials were treated with spore suspension of *Trichoderma viride* (10^6 cfu/gm) from wastepaper substrate and after that was covered by polyethylene and incubated for 42 days at the optimal growth conditions. The produced biomass (bioprocessed materials) was used for soil treatment (at rate of 2%) for suppression of damping off of pea plants caused by *Pythium debarayanum* (soil was inoculated with the phytopathogenic fungi as described by Belal *et al.*, 1996).

Statistical analysis:

The obtained data were subjected to the proper statistical procedures for analysis of variance according to Gomez and Gomez, (1984).

RESULTS AND DISCUSSION

Seven different sources (mushroom fruits, wastepaper, soil, mature compost, primary effluent mud, waste water) were used to isolate the cellulolytic microorganisms in the present study. One hundred microorganism were isolated on MSA by using clear zone formation on agar plates. Sixteen cellulylotic microorganisms from the 100 isolated (comprising fungi and bacteria) as potential cellulase producers were selected (Table 1). A preliminary classification based on the morphology of the isolates revealed that the wastepaper – degrading microorganisms belong to the group of fungi as well as to the group of bacteria. Thirteen fungal isolates of 16 were isolated and identified as *Trichoderma viride, T. harzianum, T. reesei, Penicillium* sp., *Rhizopus* sp., *Mucor* sp., *Aspergillus niger, Fusarium solani, Acremonium strictum, Cladosporium herbarum, Pleurotus* sp., *Agaricus* sp. and *Myrothecium* sp. Fungi are well-known agents of decomposition of organic matter in general and cellulose substrates in particular (Lynd *et al.*, 2002).

Three of 16 wastepapers – degrading organisms were bacteria. Two of 3 were gram- positive, non-motile, filamentous and identified as *Streptomyces* sp. and *Microbispora* sp.

One of 3 bacterial isolates was gram -positive, motile, rods, and spore former and identified as *Bacillus* sp.. Obviously, fungi play an

outstanding role in degrading of the tested wastepapers materials, since the majority of strains belong to this group. It is known that many genera of fungi play an important role in degradation of anthropogenic substrates. Due to the paucity of growth which was generally observed on MSA + carboxymethyl cellulose. The bacterial isolates were also routinely streaked onto plates of TSB or nutrient agar for bacterial strains but the fungal strains were further purified by using acidic complex medium (PDA) or addition of ampicilline 800mg/l to complex medium (PDA).

Results in Table 1 showed that the strains were tested for their growth ability on MSL supplemented with wastepaper materials (foolscap, filter paper (whatman paper No.1), cardboard (packing materials), tissue paper and newspaper) as a sole source of carbon. The general trend of biodegradability with all strains of carboxymethyl cellulose was > filter paper > foolscap > cardboard > tissue paper > newspapers. carboxymethyl cellulose, filter paper and foolscap exhibited the highest degree of bioconversion followed by the other materials because the diameter of clear zone value was wider than the other materials. Carboxymethyl cellulose and filter paper were a more favourable carbon source for screening the cellulolytic microorganisms. On the other hand, newspaper exhibited the lowest degree of bioconversion by the cellulolytic microorganisms and this may be depend on cellulose type (amorphous or crystalline) acting on the organism (Ortega *et al.*, 2001). The obtained results show also that the fungal strains exhibited the highest biodegradability for the wastepaper than the bacterial strains.

Among 16 isolated strains, one strain was identified as *Trichoderma viride* exhibited relative high clearing of plates which was supplemented with carboxymethyl cellulose as substrate for cellulase. This indicates that this strain is the highest degradability for the tested wastepaper materials than the other strains. The obtained results were compared with the growth of the isolates in MSL (no wastepaper materials). *Trichoderma* is known as a very good producer of cellulases, perhaps due to the different adaptability of fungi to the anthropogenic substrates and different resistance to the factors affecting fungal populations during the recycling procedures. Our results are in agreement with previous findings reported by Teather and Wood, (1982); Bradner *et al.*, (1999) and Peciulyte (2007).

Therefore, *Trichoderma viride* as efficient for productivity of extracellular cellulase was selected for the further studies.

Table (1): Degradation of different wastepaper materials by the microorganisms via measuring clear zone.

Microorganisms	Diameter of clear zone (mm)					
	Carboxymethyl cellulose	Filter paper	Foolscap	Cardboard	Tissue paper	Newspapers
Trichoderma viride	40	36	29	19	13	10
T. harzianum	32	26	20	16	10	8
T. reesei	33	27	21	15	11	7
Penicillium sp.	27	23	22	13	10	7
Rhizopus sp.	23	20	17	12	10	7
Mucor sp.	29	24	16	11	9	9
Aspergillus niger	28	23	18	12	8	8
Fusarium solani	27	21	16	11	9	7
Acremonium strictum	26	20	18	11	9	7
Cladosporium herbarum	23	18	12	11	10	7
Pleurotus sp.	37	28	23	18	12	9
Agaricus sp.	36	29	22	17	11	8
Myrothecium sp.	23	16	13	11	9	8
Streptomyces sp.	22	17	12	10	9	7
Microbispora sp.	21	13	12	11	8	7
Bacillus sp.	18	12	11	9	7	7

Effect of pH and temperature on growth of *Trichoderma viride* and its cellulase (CMCase) production

Environmental factors do not only influence the wastepapers to be degraded, they also have a crucial influence on the microbial population and on the activity of the different microorganisms themselves and also the amount of the enzyme production depends on the biomass. Factors such as temperature and pH, have important effects on the microbial degradation of wastepapers and so these conditions must be considered when the biodegradability of wastepapers is tested. Karpouzas and Walker (2000) reported that the degradation of ethoprophos by *Pseudomonas putida* strains epl and II affected by pH and temperature. The question is now, what are the optimal conditions (pH and temperature) for the growth of *Trichoderma viride* and its cellulase (CMCase) production?. To

determine the optimal growth conditions, carboxymethyl cellulose was used as a sole source of carbon.

Optimum pH:

The influence of pH on biomass yield of *Trichoderma viride* and its cellulase (CMCase) production is shown in Fig. 1. Generally, the optimum pH was 6.5 for *Trichoderma viride*. The maximum mycelial dry weight for *Trichoderma viride* and its cellulase (CMCase) production were recorded at pH6.5. *Trichoderma viride* grew at quite wide pH range (from 4 to 8). This variation is very useful to use these isolates in degradation test in different environments at different pH. Therefore, it can expect that these isolates can tolerate the pH change during the degradation process thereby increase the degradation potential for these isolates.

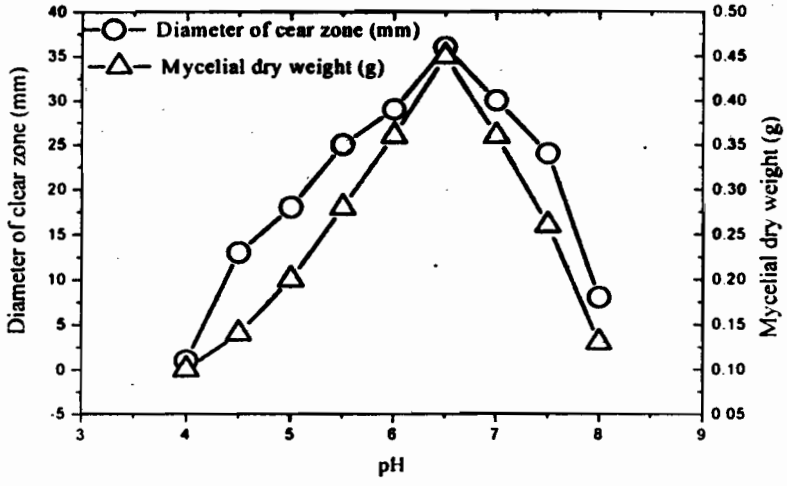

Fig.(1): Effect of pH on growth of *Trichoderma viride* and its cellulase (CMCase) production by measuring clear zone.

Optimum temperature:

The effect of different temperatures on growth of *Trichoderma viride* and its cellulase (CMCase) production is shown in Fig.2. respectively. A temperature 25°C appears to be the optimum for growth of *Trichoderma viride* and its cellulase (CMCase) production. *Trichoderma viride* and its cellulase (CMCase) production exhibited growth and cellulase (CMCase) production at different temperatures but *Trichoderma viride* did not grow at 40°C. Therefore, these strain was used for further studies under the optimum growth conditions with

the aim of the effect of different carbon source on growth *Trichoderma viride* and enzyme induction as well as determination of the degradation potential for the wastepaper material under solid state fermentation.

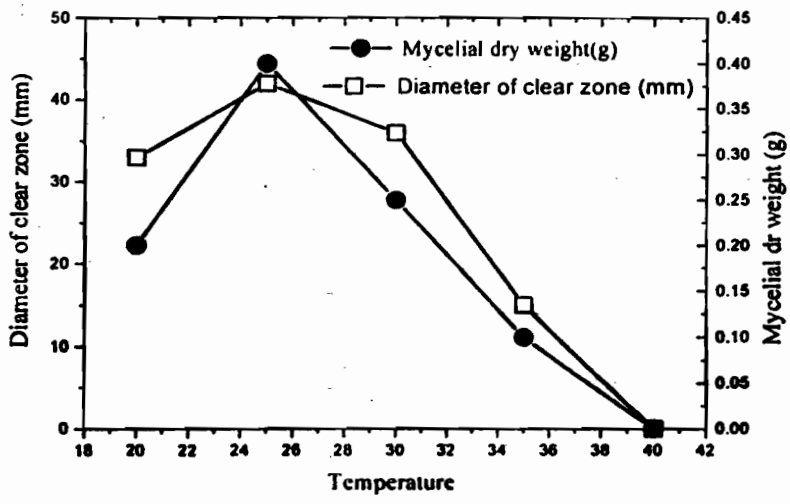

Fig.(2) : Effect of temperature (°C) on growth of *Trichoderma viride* and its cellulase (CMCase) production by measuring clear zone.

Regulation of enzyme production (constitutive or inductive enzyme)

According to Schlegel (1992) most enzymes systems involved in substrate degradation are inductive enzymes. Therefore it is of interest to know, if the wastepaper degrading enzyme system is constitutively secreted or induced by the presence of wastepaper or other carbon source. Furthermore it is of interest, if the enzyme is inducible, what are the substances inducing the enzyme activity?

Fig. 3. shows that the fungal growth (determined as mycelial dry weight) was high on PD, followed by MSL+glucose, MSL + carboxymethyl cellulose, MSL + filter paper, MSL + foolscap, MSL + cardboard, MSL + tissue paper, MSL + newspaper and latter on MSL without carbon source.

Fig. 3.and Fig.4. indicate that the extracellular cellulase was produced only during growth of *Trichoderma viride* on all wastepaper materials in MSL medium as carbon sources. The results demonstrated

that a maximum cellulase activity was obtained when carboxymethyl cellulose followed by filter paper, foolscap, cardboard, tissue paper were used as substrate. The low enzyme activities measured with newspapers. On the other hand in PD as a complex medium or in MSL+glucose, enzyme secretion is not induced despite the media generated a good cell growth. This results are in agreement with my previous findings and other investigators while secretion of PCL-hydrolase was only induced in the culture supernatant with PCL as aliphatic homopolyester or BTA 45:55 (Ecoflex) as copolyester as substrates but was not induced on glucose or GYM as complex medium (Lin and Kolattukudy (1978), Murphy *et al.*, (1996), Belal 2003).

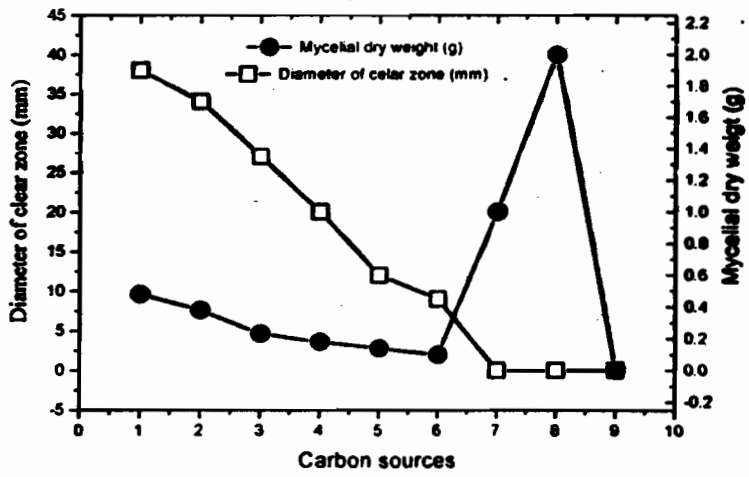

Fig.(3): Effect of different carbon sources on growth of *Trichoderma viride* and its cellulase (CMCase) induction, where: 1 -carboxymethyl cellulose, 2-filter paper, 3-foolscap, 4-cardboard, 5-tissue paper, 6-newspapers, 7-glucose, 8-PD as complex medium, 9-MSL medium plus *T. viride* without any carbon source.

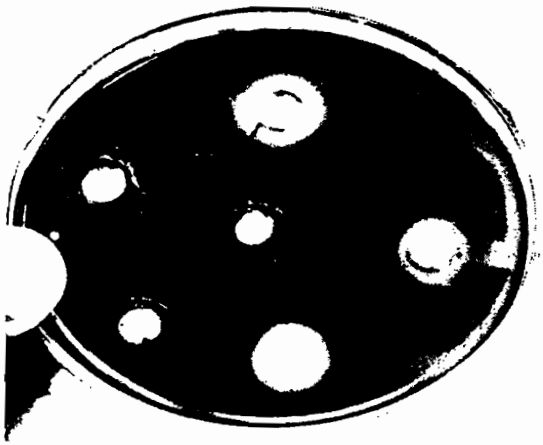

Fig.(4). Determination of cellulase activity produced by *Trichoderma viride* via clear zone formation on MSA supplemented with carboxymethyl cellulose, where: A (foolscap), B (cardboard) and C (tissue paper) clear zone formation and D (MSL medium without any carbon source plus *T. viride*), E (MSL medium with glucose) and F (PD medium) no clear zone formation.

Saccharification of wastepapers materials by the *Trichoderma viride* cellulase

Aside from the traditional methods of waste management, biowaste has been used in the production of clean energy where it replaces coal, oil or natural gases to generate electricity through combustion. The conversion process of wastes to energy has been proved to be safe, environmental friendly and reduces the incoming volume of waste to a great extent. An alternative to the combustion of biowaste could be through the fermentation of saccharified waste cellulose into bioproducts.

An initial increasing trend of sugar formation was observed when more of each wastepaper substrate was degraded with a fixed enzyme concentration (Table 2). Carboxymethyl cellulose and filter paper showed more bioconversion than the other paper materials. The trend of biodegradadability with *T. viride* cellulase of carboxymethyl cellulose was > filter paper > foolscap > cardboard > tissue paper > newspapers. Due to the structural composition of wastepaper material it can be biodegraded into fermentable sugars. The variation in wastepaper bioconversion by *T. viride* cellulase could be due to the composition of the enzyme system as well as the structure of cellulose. this consists of a crystalline section. which is difficult to hydrolyze. and an amorphous section that is more susceptible to cellulase attack (Van

Wyk and Mohulatsi, (2003). The present study showed also that, the trend of biodegradadability of different wastepaper materials with cellulase was more than the trend of biodegradadability of different wastepaper materials by *T. viride* because after14days of cultivation *T. viride*, the production of reducing sugar was almost (enzymatic activity measured-by the production of reducing sugars end group, which is taken to be an indication of cleavage of cellulose molecules) equal to which produced with cellulase after the incubation period (at 50°C for 30min).

It is of interest that isolation and purification as well as characterization of *T. viride* cellulase in the next study and using it in different industrial purposes as well as in bioconversion other cellulolytic materials such as agricultural wastes.

In most investigations, members of the fungal genus *Trichoderma* have been extensively studied due to their ability to secrete cellulose-degrading enzymes. Most of the works have been carried out on *T. aureoviride* Rifai, *T. viride* Pers., *T. reesei* E. G. Simmons, *T. harzianum* Rifai strains and their mutants evaluating their ability to produce extracellular cellulolytic enzymes (endoglucanases, exoglucanases and cellobiase) which act synergistically in the conversion of cellulose to glucose. The cellulases secreted by *Trichoderma* have received widespread industrial interest leading to commercial applications (Olson and Hahn-Hagerdahl (1997); Oksanan *et al.*, (2000); Mach and Zeilinger (2003); Cavaco-Paulo and Gübitz, (2003), Nierstrasz and Warmoeskerken, (2003); Van Wyk and Mohulatsi, (2003); Penttila *et al.*, (2004).

Table (2) Degradation of different wastepaper materials by *Trichoderma viride* cellulase

Wastepaper materials	Activity of cellulase (U/ml/min) after the incubation period (at 50°C for 30min)	Activity of *T. viride* cellulase (U/ml/min) after the incubation period (14 days at 25°C and 150 rpm)
Carboxymethyl cellulose	3.3	3
Filter paper	2.2	2.1
Foolscap	1.7	1.6
Cardboard	1.2	1.1
Tissue paper	0.7	0.6
Newspapers	0.4	0.3

Biodegradation of different wastepaper materials by *Trichoderma viride*

Weight loss determination of wastepaper on agar plates

The degradation potential of *Trichoderma viride* was quantified (expressed as weight loss (mg), % degradation, and degradation rate per surface area [mg/(week cm²)] for wastepaper films on agar plates after 3 weeks incubation at 25°C (Table 3). *Trichoderma viride* grew on mineral salts agar plates containing wastepaper films (filter paper, foolscap, cardboard, tissue paper and newspapers) as a sole source of carbon (Fig.5).

The obtained results shows that, filter paper and foolscap films were degraded much faster than the cardboard, tissue paper and newspapers films which were a more favourable carbon source for *Trichoderma viride*. It was also observed that the incubation time was longer than the previous experiment and this due to the surface area of the wastepaper materials.

Table (3) Degradation rate [mg/(week cm²)] of different wastepaper materials by *Trichoderma viride* on agar plates

Wastepaper materials	% Degradation	Degradation rate[mg/(week cm²)]
Filter paper	100	1.7
Foolscap	100	1.4
Cardboard	75	1.02
Tissue paper	45.7	0.54
Newspapers	25	0.34

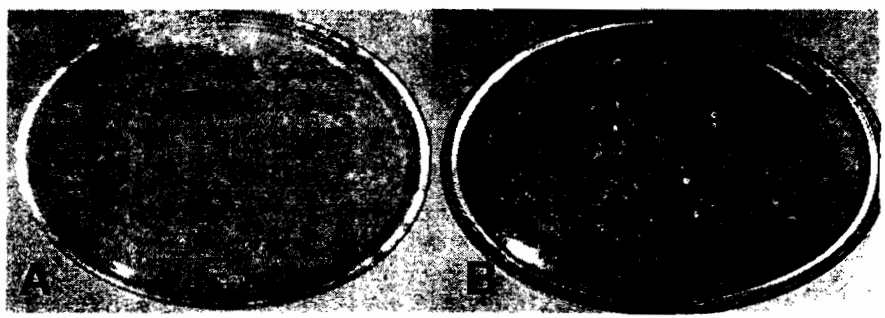

Fig.(5). Degradation of filter paper films by *Trichoderma viride* on agar plates where: A: Control, and B: inoculated with *Trichoderma viride*

Biodegradation of mixture of wastepaper materials by *Trichoderma viride* by using solid state fermentation and using the bioprocessed materials in suppression of damping-off caused by *Pythium debarayanum*

It is of interest to mix wastepaper materials together with removing of newspapers(because it containing lead) and degradation of the wastepaper mixture by *Trichoderma viride* in solid state fermentation. A mixture of wastepaper materials exhibited completely degradation after 6 weeks and color of mixture of wastepaper materials convert to *Trichoderma viride* color (green color) and this produced biomass was named as (bioprocessed materials). The produced biomass (bioprocessed materials) contained high numbers of from *Trichoderma viride* which contained (10^5cfu/ml).

It was of a particular interest to use the produced biomass (bioprocessed materials) as biocontrolling agent for suppression of damping-off of pea caused by *Pythium debarayanum*, since, different species of *Trichoderma* were previously used in controlling of *Pythium* seed rot and *Rhizoctonia* root rot in different crops such as pea (Harman *et al.*, 1980, Nelson *et al.*, 1988 and Belal *et al.*, 1996).

Under greenhouse conditions, application of the bioprocessed materials to soil infested artificially with *Pythium debarayanum* (Table 4) exhibited their efficacy to control damping-off of pea and increased survival plants. The effects were similar in most cases to those of maxim fungicide. Also, Attempts had been successfully carried out using antagonists to control soil – borne fungal pathogens on pea (Harman *et al.*, 1991, Belal *et al.*, 1996, Mao *et al.*, 1997, Xue, 2001 and 2003).

Table (4) Biocontrol of damping-off of pea by the bioprocessed materials.

Treatments	% pre-emergence damping-off	% post-emergence damping-off	% survival plants
Control (un-inoculated)	0a	0a	100a
Pythium debarayanum	60b	20b	20b
T. viride + *Pythium debarayanum*	10c	5c	85c
Maxim+ *Pythium debarayanum*	5c	5c	90c

Values having the same alphabetical letter within column are not significantly different ($P < 0.05$).

The obtained results indicate the necessity of biodegradation of wastepaper and use of the bioprocessed materials as alternative for fungicides to reduce the environmental pollution which caused by wastepaper materials or chemical fungicides.

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الملخص العربي

التكسير الحيوي للمخلفات الورقية باستخدام *Trichoderma viride* واستخدام المواد التي تم تدويرها (bioprocessed materials) في المقاومة الحيوية لمـرض تساقط البادرات في البسلة المتسبب عن *Pythium debarayanum*

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تم دراسة التكسير الحيوي للمخلفات الورقية مثل ورق الترشيح والفلوسكاب والكرتون وورق المناديل وورق الجراند. باستعمال طريقة الهالة الشفافة بالإطباق ، تم عزل ما يقرب من ١٠٠ سلالة ميكروبية (شاملة الفطريات والبكتيريا) من مصادر مختلفة للميكروبات باستعمال مادة كربوكسي ميثيل سيليلولوز كمادة تفاعل لإنزيم السيليلوليز. تم تحديد ١٦ من الميكروبات المحللة للسيليلولوز (شاملا الفطريات والبكتيريا) من ١٠٠ التي تم عزلها والتي كانت أكثر قدرة على أنتاج إنزيم السيليلوليز.

تم اختيار سلالة فطرية واحدة من ١٦ السلالة المحللة للسيليلولوز والتي أعطت أكبر هالة شفافة عن السلالات الأخرى. وتم تعريف هذه السلالة على أنها *Trichoderma viride*. وقد أوضحت النتائج إن رقم الحموضة 6.5 وأيضا درجة الحرارة ٢٥° مئوية هما المثلى لنمو فطر *Trichoderma viride* وأيضا إنتاجية إنزيم السيليلوليز.

أظهرت النتائج المتحصل عليها من هذه الدراسة أن إنزيم السيليلوليز المنتج من *Trichoderma viride* يحفز في مزرعة النمو في وجود مادة كربوكسي ميثيل سيليلوليز ومخلفات الورق (ورق الترشيح والفلوسكاب والكرتون وورق المناديل وورق الجراند) في البيئة التركيبية (MSL) ولكن في حالة وجود مصدر آخر للكربون مثل الجلوكوز أو البيئة الكاملة مثل بطاطس الدكستروز ((Potato Dextrose (PD) تثبط إنتاج إنزيم السيليلوليز.

أظهرت النتائج المتحصل عليها أن كل مخلفات الورق أظهرت مدى قابليتها لإنزيم السيليلوليز لتحويلها إلى سكريات مختزلة. وكانت القدرة التحليلية لإنزيم السيليلوليز أعلى من القدرة التحليلية فطر *Trichoderma viride*.

إضافة لاختبار طريقة الهالة الشفافة بالإطباق تم تقييم القدرة تحليلية لفطر *Trichoderma viride* لكل مخلفات الورق وذلك بتقدير الفقد في الوزن. حيث أظهرت الدراسة أيضا أن فطر *Trichoderma viride* أظهر قدرة تحليلية لكل مخلفات الورق سواء كانت على بيئة أجار (MSA)في أطباق وذلك بتقدير الفقد في الوزن. كما أظهر أيضا قدرة تحليلية لخليط مخلفات الورق مع بعضها (مع استبعاد ورق الجراند) مستخدما تكنيك تخمر المواد الصلبة مؤديا لتكوين الكتلة الحيوية(bioprocessed materials).

كما أظهرت النتائج بأن معاملة التربة ب (bioprocessed materials) كانت فعالة ومؤثرة في مقاومة مرض تساقط بادرات البسلة المتسبب عن *Pythium debarayanum* وذلك

مقارنة بالمعاملات الغير معاملة (كنترول). وكان التأثير مشابه إلى حد كبير إلى تأثير المبيد الفطري الماكسيم على المسبب الممرض حيث كانت نسبة النباتات الباقية غير مختلفة معنويا في الحالتين.

نستخلص من نتائج هذه الدراسة إمكانية استخدام المواد التي تم تدويرها (bioprocessed materials) وذلك لتقليل التلوث البيئي الناجم عن المخلفات الورقية وأيضا كبديل للمواد الكيماوية المستعملة في مقاومة تساقط بادرات البسلة المتسببة عن *Pythium debarayanum*