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MANAGEMENT OF DAMPING-OFF AND ROOT–ROT OF SUGAR BEET USING BIOCONTROL AGENTS GROWN ON SOME PLANT WASTES

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ABSTRACT: ***Corn cobs/ sugar cane bagasse /rice straw and wheat straw were individually used as carbon sources in Czapek-Dox medium to cultivate either Trichoderma viride or Trichoderma lignorum in order to control root-rot disease of sugar beet. The antifungal activities of crude extracts of both biocontrol agents were in vitro determined against Rhizoctotonia solani, Fusarium exysporum and Sclerotiun rolfsii. High amounts of chaetocin and gliotoxin were produced in rice straw medium, while chitinase production was higher in sugarcane medium. Under greenhouse and artificial soil infestation conditions, both Trichoderma isolates significantly reduced root-rot infection especially those grown on Dox-rice straw and Dox-sugarcane bagasse in compared with untreated control (pathogen only).***

In general, the tested media costs were lower than Czapek Dox one. In addition, the use of these wastes will minimize their hazard effects on the environment.

Key Words*: Biological control, Root-rot, Sugar beet, Trichoderma, Carrying, Materials, Enzymes and toxins.*

INTRODUCTION

Sugar beet (*Beta vulgaris*) is one of the most important crops in Egypt. It is subject to serious diseases, especially root–rot which causes losses in root, sugar yield and purity%, (Manal, 2005).

Biological control of soil –borne plant pathogens is a potential alternative to the use of chemical pesticides, which are harmful to the environment. Fungal biocontrol agents, including the extensively studied *Trichoderma* spp. have been widely used as antagonistic fungal agents against several pests as well as plant growth enhancers (Verma *et al*. 2007). However, sugar beets are susceptible to a number of seedling and root rot diseases that are primary constraints to profitable sugar beet production (Harveson 2006). *Trichoderma viride* and *Trichoderma lignorum* in addition to other weakly virulent or saprophytic fungi, have been reported to reduce infection or reproduction of many pathogens. The fungi are relatively easy to grow and formulate for large–scale application and several are now available commercially (Punga 1997). Several strains of the fungi *Trichoderma viride* and *Trichoderma lignorum* have been isolated from soil-borne plant pathogenic fungi under greenhouse.The low rate of digest ability of rice

straw is attributed to the slow rate of fermentation (Shashirekha *et al.*,2002).

***Trichoderma* spp. are used to contol *Pythium* (Khare *et al.* 2010), *Fusarium* (Abu-Taleb and Al-Mousa 2008.) and *Sclerotium* (Shaigan *et al.* 2008).**

This work was undertaken to improve the potential of certain fungi in biological control of damping-off and root rot of sugarbeet in Lab and greenhouse using four cellulosic wastes.

MATERIALS AND METHODS

1. In vitro study

Fungal strains :

***Trichoderma viride* and *Trichoderma lignorum* were isolated from the rhizosphere of sugar beet plants collected from Kafr El-Sheikh Governorate.**

Media and growth conditions:

Culture media

Potato dextrose agar (PDA), Difco, 1984 was used for cultivation of cultures. Stock cultures were maintained on Czapek Dox medium (CDA) Difco, 1984 slant, kept in refrigerator at 5-6^{0}C and were subsequently recultured on the same medium every two weeks.

Fermentation media:

Four modified Czapek Dox media were used as fermentation media. The medium was modified by adding different agricultural wastes (40g/l) as a sole carbon source instead of sucrose. The modified media were: Dox corn cobs, Dox sugarcane bagasse , Dox rice straw and Dox wheat straw. Erlenmeyer conical flasks 250 ml each containing 50 ml fermentation medium were inoculated with the biocontrol agent then incubated on a rotator incubator shaken at 180 rpm for 2 weeks at 28-30^{0}C .

Extraction of toxin:

The culture filtrates were adjusted to pH 5.1 and extracted three times with 250 ml ethyl acetate/liter. The aqueous layer was removed and the solvent layer was washed three times with 35ml of 5% aqueous sodium bicarbonate solution. The solvent layer was concentrated under vacuum till dryness and re-dissolved in ethyl acetate (Sawai *et al.*, 1980).

Determination of toxins:

Chemical analysis

Toxins were determined by thin layer chromatography (TLC).Crude samples were spotted 2cm from the bottom on a pre-coated aluminum sheet of silica gel 60 Rf_{254} (Merck). The plates were subjected to different solvent systems were expressed in volume ratio as follows: chloroform: methanol: acetic acid (90:9:1), chloroform: methanol: acetic acid (90:6:4) (Tarber *et al* 1977), chloroform: : methanol: acetic acid (92:6:2) (Sawai, 1980), benzene:

methanol: acetic acid (18:1:1) (Ahmed, 2000) and petroleum ether: chloroform: methanol: acetic acid (50:49:1).

Toxins spots were located by their fluorescence on the chromatogram under short and long wave UV light (254 and 366 nm) against standards. The R_f values were determined. Available pure toxins were used to prepare standard curves and were also analyzed by using UV spectrophotometer.

Bioassay of Toxins:

The Crude extracts of toxins were tested for toxicity against the pathogenic fungi *Rhizoctotonia solani, Fusarium exysporum and Sclerotiun rolfsii.* A freshly prepared suspension of the pathogenic fungus was used to inoculate three plates of Dox,s agar medium. The filter paper disk method was used (Clement, 1968 and Atalla & Nour El-Din,1993). A sample of 50 ug of the crude extracts was dissolved in ethyl acetate and applied to filter paper disk (5mm in diam.). The prepared disks were dried and firmly applied to the surface of the inoculated agar plates, then the plates were incubated at 28-30^{0}C for 48-72 hrs. Compound-free disks were served as control. The diameter of the inhibition zone around each disk was measured in mm.

Assay of chitinase activity:

Colloidal chitin was prepared from chitin powder (Sigma Co.) according to the method described by Reid and Ogrydziak (1981). Twenty five grams of chitin powder suspended in 250 ml of 85%phosphoric acid (H_3PO_4) and stored at 4^{0}C for 24 h., then blended in 2 liter of distilled water, using a wiring blender, and centrifuged. The washing procedure was repeated twice. The colloidal chitin suspension was adjusted to pH 7.0 with (IN) NaOH and re-centrifuged. The pelletted colloidal chitin was stored at 4^{o}C until used.

Determination of enzyme activity was carried out according to the method of Monreal and Reese (1969). One ml of 1% colloidal chitin in 0.05 M citrate phosphate buffer (pH 6.6) was mixed with one ml of enzyme extract by shaking. Reaction mixture tubes were incubated in water bath at 37 ^{o}C for minutes, then cooled and centrifuged before assaying. Reducing sugars were determined as ml of supernatant by dinitro- salysilic acid (DNS). The optical density was determined at 540 nm.

2. In greenhouse study

Pathogen inocula:

***Rhizoctonia solani, Fusarium exysporum and Sclerotiun rolfsii* were grown separately in natural corn medium for 15 days at 30^{o}C ±2 ^{o}C. An inoculum was added to soil at a rate of 3 g/100g soil.**

Preparation of the bioagent:

***T. viride* and *T . lignorum* were separately grown on the best selective medium (in- vitro test). Dox- rice straw, Dox-sugarcane bagasse, Dox wheat straw and corn cobs for 2 weeks and applied to the previously infested soil with the pathogenic fungus. Uninfected treatments were also prepared. The**

percentage of damping–off was recorded as pre- and post-emergence respectively after 7and30 days from sowing slate. Disease severity of root rot was estimated after harvest, according to 0-7index; Engelkes and Windels (1996).

RESULTS AND DISCUSSION

I. Laboratory experiments:

a. Influence of medium on toxin production and chitinase activity:

Gliotoxin production and chitinase activity of *Trichoderma . viride* and *Trichoderma . Lignorum* were studied in triplicate for each strain. From the results in Tables (1 and 2), it is obvious that the amount of gliotoxins increased to (85.40 and 70.52 mg/l, respectively) when *T. Lignorum* and *T. viride were* grown in a Dox-rice straw medium. *T. Lignorum* and *T. viride* produced variable amounts of chitinase enzyme in all different media used. Data also revealed that Dox-sugarcane bagasse medium was most favorable for chitinase production (1.48U/ml by *T. Lignorum* and 1.28U/ml by *T. viride*). Generally, Dox-rice straw and Dox sugar cane bagasse had the highest effects on both studied Toxins .In contnast ,Dox corn cobs gave the least effect.The results show the possible usage of inexpensive waste materials for toxins production and enzyme activity (Hamed, 2001).

Table (1): Effect of different fermentation media on chitinase activity and gliotoxin production by *Trichoderma lignorum*

Fermentation media	Chitinase activity(U/ml)	oxin(mg/l) Gliot
Dox-corn cobs	0.32	19.22
Dox-sugarcane bagasse	1.48	62.77
Dox-rice straw	0.96	85.40
Dox-wheat straw	0.40	50.32

Table (2): Effect of different fermentation media on chitinase activity and gliotoxin production by *Trichoderma viride*

Fermentation media	Chitinase activity(U/ml)	oxin(mg/l) Gliot
Dox-corn cobs	0.40	16.21
Dox-sugarcane bagasse	1.28	58.67
Dox-rice straw	0.66	70.52
Dox-wheat straw	0.40	36.14

b. Antifungal activity of crude extracts of *T. Lignorum* and *T. viride toxins:*

The inhibitory effects of crude extracts of *T. Lignorum* and *T. viride* toxins against pathogenic fungi (*Rhizoctonia solani, Fusarium exysporum and Sclerotium rolfsii*) are shown in Table (3) crude extract of *T. Lignorum* toxins from Dox-rice starw was highly active against *Rhizoctonia solani ,Fusarium exysporum and Sclerotiun rolfsii* as indicated by the diameter of the

inhibition zone (3.5, 2.6 and 2.0 mm, respectively). Similar results were obtained from toxins extract of *T. viride* against *Rhizoctotonia solani, Fusarium exysporum and Sclerotiun rolfsii* . the crude extract of *T. Lignorum* had higher effect than those of *T. viride*

The major constraint of using rice straw as feed is its high lignin and silica content, low protein and low digestibility (Jackson, 1978). *T. lignorum* was a good source of complex cellulose enzymes (Cl+Cx enzyme) for enzymatic hydrolysis of all examined substrates (El-Zawahry and Mostafa, 1983). Papavizas (1985) revealed that *T. lignorum* and *T. viride* are good source of various enzymes such as exo and endo-glucanases cellobiase and chitinase. Tronsmo and Harman (1992) found that addition of any of several complex materials to the fermentation medium of *T. harzionum* increased hydrolytic enzymes, so Dox medium supplemented with rice straw is favorable for the growth of *T. lignorum* and *T. viride*.

Table (3): Antifungal activities of crude extracts of *Trichoderma lignorum* and *Trichoderma viride* toxins in different fermentation media against pathogenic fungi.

Pathogen fungi / Fermentation media	Diameter of inhibition zone in (mm)		
	Rhizoctotonia solani	*Fusarium exysporum*	*Sclerotiun rolfsii*
	Trichoderma lignorum		
Dox-corn cobs	0.5	0. 5	0.3
Dox-sugarcane bagasse	3.0	2.0	1.5
Dox-rice straw	3.5	2.6	2.0
Dox-wheat straw	2.1	1.6	0.8
Control	0.0	0.0	0.0
	Trichoderma viride		
Dox-corn cobs	0.3	0.4	0.3
Dox-sugarcane bagasse	1.6	1.0	0.7
Dox-rice straw	2.0	1.4	1.0
Dox-wheat straw	1.3	0.8	0.5
Control	0.0	0.0	0.0

II. Greenhouse experiments:

Effect of bioagents grown on agricultural wastes on disease severity of root rot of sugar beet :

Root–rot disease caused by soil borne fungi are the most serious disease of sugar beet plants (El-kholi;2000; Gouda, 2001 and Manal, 2005).

Data in Table (4) indicate that the two antagonistic fungi grown on rice straw and sugarcane bagasse significantly reduced the percentage of infection with root-rot especially in case of *T.viride* + rice straw with *R. solani* or + sugarcane bagasse with *F. exysporum* or *Sclerotiun rolfsii*.

As whole, it could be concluded that Dox –sugarcane bagasse medium , *in vitro,* produced the highest chitinase activity followed by Dox rice straw. The last medium had the highest Gliotoxin production followed by Dox

sugarcane bagasse. The most effective medium on *F. exysporum* or *S. rolfsii* and *R. solani* was Dox rice straw followed by Dox sugarcane bagasse in Lab. However, under greenhouse conditions, *T. Lignorum* and *T. viride* with Dox rice straw had the highest effect on the tested damping-off and root rot diseases. On the other hand, it could be used the previous antifungal agents with Dox sugarcane bagasse for control *F. exysporum* or *S. rolfsii*.

Table (4): Effect of biocontrol-agents grown on different fermentation media in controlling damping-off and root rot disease of sugar beet under greenhouse conditions

Treatments	Fungus											
	Rhizoctotonia solani				*Fusarium exysporum*				*Sclerotiun rolfsii*			
	*Pre	*Post	*Surv	*D.s	*Pre	*Post	*Surv	*D.s	*Pre	*Post	*Surv	*D.s
T. lignorum with corn	22.2	35.6	42.2	3.0	2.5	28.7	41.0	30.3	12.5	33.3	54.2	3.5
T. viride with corn	19.5	31.0	49.5	2.6	2.0	33.6	38.3	28.1	15.0	28.4	56.6	3.0
T. lignorum with rice straw	9.2	20.1	70.7	1.0	1.0	64.3	21.2	14.5	7.5	21.0	71.5	1.6
T. viride with rice straw	12.0	25.2	62.8	1.7	1.3	61.1	25.8	13.1	8.7	26.0	65.3	2.2
T.lignorum with sugarcane bagasse	15.4	30.0	54.6	2.0	1.5	53.5	29.4	17.1	11.2	30	58.8	3.2
T. viride with sugarcane bagasse	20.1	37.6	42.3	2.6	2.0	48.8	33.2	18.0	13.0	30	57.0	4.0
Control (fungi alone)	33.0	55.5	11.5	4.5	5.5	1.0	55.0	44.0	42	55	3.00	6.0

LSD at 5%	Pre.	Post.	Surv.	D.s.
Fungus A	0.02	0.29	2.94	0.06
Treatment B	0.02	0.43	3.26	0.10
AxB	0.04	0.75	5.64	0.18

***pre = pre-emergence damping-off %**
***post = post- emergence damping-off %**
***surv = survival plant%**
***D.S = disease severity%**

Chiu and Huang (1997) used different composts as culture media for the occurrence of watermelon fusarial wilt, cabbage club root,and root-knot nematode of watermelon. These media are also able to suppress Rhizoctonia damping of cabbage, watermelon and pepper. Therefore, they play an important role for controlling disease.

These results are in line with those of Nwodo-Chinedu *et al.* (2007), who found that modified Czapek-Dox agar containing sugarcane pulps gave 87.8 – 93.8% of the maximum growth obtained on Sabouraud's agar. The modified Sabouraud's agar containing sugarcane pulps yielded 94.4 – 100% of the maximum growth on Sabouraud's agar.

Dissanyake and Hoy (1999) found that all organic materials contained increased levels of organic C and total N and elevated levels of other nutrients when compared with field soil. A group of materials that best suppressed root-rot and increased plant growth when added in non sterile form, may bedue to the relationship of mineral nutrient levels in organic materials capable of suppressing soil borne disease. In addition, it is favorable for the growth of antagonistic fungi and toxins and induce enzymes production.

Generally, the utilization of the pervious cellulosic wastes in media formulations would definitely reduce the microbial media cost. Besides, this would offer a means of transforming the vast quantities of waste cellulosic materials available in our environment into useful products and at the same time reduce the problem of waste disposal.

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مكافحة مرض موت البادرات وعفن الجذور في بنجر السكر باستخدام كائنات تضاد حيوي منماه على بعض المخلفات النباتية

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الملخص العربي

يهدف هذا البحث إلى استخدام بعض المخلفات الزراعية مثل قوالح الذرة، مصاصة القصب، قش الأرز وقش القمح كمصدر كربوهيدراتى فى بيئة تشابك دوكس لتنمية الفطرين تريكودرما فيردى وتريكودرما ليجنورم واستخدامهما في مكافحة مرض موت البادرات وأعفان الجذور في نبات بنجر السكر. وأثبتت النتائج أن البيئة المضاف إليها قش الأرز هي الأفضل في إنتاج السموم المضادة لكلا فطرى ترايكودرما. أما تلك المستخدم فيها مصاصة القصب فكانت الأنسب لإنتاج أنزيم كيتينيز. وتحت ظروف الصوبة، أثبت فطرى تريكودرما فعالية في مكافحة مرض موت البادرات وعفن الجذور بصورة معنوية، وكانت أفضل النتائج المتحصل عليها تلك المستخدم فيها قش الأرز وقش القمح ومصاصة القصب مقارنة بتلك المنماة على الذرة أو الغير معاملة (الفطر الممرض فقط).