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Trichoderma asperellum isolated from salinity soil using rice straw waste as biocontrol agent for cowpea plant pathogens

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ABSTRACT

This study discusses isolation and identification new fungal isolate from salinity soil for controlling soil borne diseases. Among sixteen fungal, a potent isolate coded SRBP_ZSHSG1 was isolated from *Sugar beet* rhizospher samples collected from Al-Hosainia localities-El-Sharkia-Egypt. Traditional methods consistent with phylogenetic analysis of 18S rRNA sequences showed SRBP_ZSHSG1 has 100% similarity with *Trichoderma* strains and the most closest is *Trichoderma asperellum.* Thus, it proposed name *Trichoderma asperellum* SRBP_ZSHSG1 (ID: KP336489). Results proved SRBP_ZSHSG1 followed by *T. roseum* and *Chaetomium globosum* were highly inhibitors to the tested pathogens. These results were confirmed by field experiments. SRBP_ZSHSG1 was able to grow on rice straw (biostraw) and produce most active compounds. The biostaw extract was the most effective bioagent and recorded highest reduction in pathogen numbers. GC/MS analysis of ethyl acetate extract revealed the presence of 9 compounds. These compounds were determined 4 volatile alcohols (1-4) and fatty acid esters (5-9).

INTRODUCTION

Soil salinity refers to the presence of high concentrations of soluble salts in the soil moisture of the root zone. These concentrations of soluble salts, through their high osmotic pressures, affect plant growth by restricting the uptake of water by the roots. All plants are subject to this influence, but sensitivity to high osmotic pressures varies widely among plants species. Salinity can also affect plant growth because the high concentration of salts in the soil solution interferes with a balanced absorption of essential nutritional ions by the plants. The main effects of salinity on plant growth and crop production are: The spread of plant pathogenic fungi which cause damping-off, wilt and root-rot diseases, agricultural soil Slow and insufficient germination of seeds, Physiologic drought, wilting, and desiccation of plants; Stunted growth, small leaves, short stems and branches; Blue-green leaf color; Retarded flowering, fewer flowers, sterility, and smaller seeds; Growth of salt-tolerant or halophilous weed plants; As a result of all these unfavorable factors, low yields of seeds and other plant parts (Parida and Das 2005). As a result, need of an hour is selection for some eco-friendly biocontrol agent that is resolving the above mentioned problems. *Trichoderma* is one such genus that is ubiquitously present in the environment which has naturally sustained the agricultural yields. *Trichoderma* are closely related with their ability to produce a wide range of lysing enzymes, to degrade substrates and to possess high resistance to microbial inhibitors (Strange 1993). *Trichoderma* sp. include a number of fungal strains that are used as biocontrol agents due to their abilities to antagonize a wide range of phytopathogenic fungi, bacteria and oomycetes, through several mechanisms that are activated in *Trichoderma* by the pathogens. *Trichoderma* sp. antagonizes phytopathogens by competing for nutrients, space, by producing antibiotics as well as by inducing systemic resistance of plants. In addition, *Trichoderma* sp. stimulates plant growth and development by means of the production of plant growth promoting molecules (Eziashi, *et al*., 2007 and Singh *et al*., 2014).

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The soil is the natural habitat of the majority of micro-organisms which produce antibiotics when grown on laboratory culture media. Some of these substances inhibit *in vitro* the germination of fungal spores and the growth of certain fungal plant pathogens. Therefore, the soil flora could be induced to form antibiotics in the soil it might be possible to control soil-borne pathogens very effectively by inhibiting their development in the soil (Cota *et al*., 2007; Barker and Panlitz 1996; Eziashi *et al*., 2007). Antibiotics substances produced by natural metabolic processes of some microorganisms that can inhibit or destroy other microorganism. A number of antibiotic drugs have been discovered from soil-inhabiting microorganisms which include fungi (20% of isolated antibiotics), actinomycetes (70%) and eubacteria (10%) (Makut & Owolewa 2011). Several fungi, were recorded as causal pathogens of root rot and wilt diseases such as *F. solani*, *F. oxysporuim*, *Rhizoctonia solani*, and *Sclerotium rolfsii* (Abou-Zeid *et al*., 1990; Abou-Zeid *et al*., 2008). To overcome the pathogenic fungi; fungicides have been used for a long time. Intensified use of fungicides has resulted in the accumulation of toxic compounds potentially hazardous to human and environment (Dekker & Georgopolous, 1982), and also in the build-up of resistance of the pathogens (Cook and Baker 1983). A reduction in chemical inputs in agriculture requires alternative methods for managing soil borne diseases for sustainable production systems. This includes the use of biological control agents (Roberts *et al.,* 2005; Spadaro and Gullino 2005; Kamal *et al.*, 2009). A large number of studies have been devoted to the identification of microorganisms able to reduce the activity of soil borne pathogens. The majority of strategies for biocontrol of such pathogens rely on a single microbial biocontrol agent for pathogen suppression (Larkin *et al.,* 1998 and Rao *et al.*, 1998). The main goal of this work is to obtain the potent fungus which able to produce natural compounds vital for disease resistance of soil, which spread dramatically in the saline soil and cause significant loss in agricultural crops.

MATERIALS AND METHODS

Source of samples and isolation of fungi from rhizospheric samples

The samples were collected from the rhizospher of *Sugar beet* samples obtained from Al-Hosainia localities- El-Sharkia Governorate- Egypt. Dilutions of the rhizosphere sample was prepared, transferred to Czapek-Dox agar plates, and incubated at 28:30 °C. Exposed to daily examination for 5 to 7 days. Fungal isolates were purified then transferred to potato dextrose agar (PDA) (Lab M Limited, Bury, Lancashire, UK) slants and incubated at 28-30 °C (Santamarina *et al*, 2002).

Identification of the isolated fungi

Phenotypic identification of the isolated fungi

The isolated fungi were identified in the National Research Center, Chemistry of Natural and Microbial Products Dept. based on their morphological characters according to Samson *et al.*(1981); Botton *et al.,* (1985); Pitt & Hocking (1985).

Genotypic identification of the isolated fungi

DNA isolation, PCR amplification and Sequencing

DNA extraction was done by using the protocol of GeneJet genomic DNA purification Kit (Thermo **# K0791**) as following manufacture of kit.

The PCR amplification of 18S rDNA region was carried out following the manufacture of Maxima Hot Start PCR Master Mix (Mix (Thermo) #K0221). The 18srDNA was amplified by polymerase chain reaction (PCR) using primers designed to amplify 1500 bp fragment of the 18SrDNA region. The ITS1–5.8S–ITS2 genomic region was amplified from genomic DNA using the forward primer ITS1 (5-TCCGTAGGTGAACCTGCGG-3) and the reverse primer ITS4 (5-TCCTCCGCTTATTGATATGC-3) (White *et al.,* 1990).

The PCR reaction was performed with 10µl of genomic DNA as the template, 1µl of 18SrRNA Forward primer, 1µl of 18SrRNA reverse primer 13 µl Water, nuclease-free and 25 µl Maxima® Hot Start PCR Master Mix (2X) in a 50µl reaction mixture as follows: activation of Taq polymerase at 95 °C for 10 minutes, 35 cycles of initial den. 95°C for 10 min, dent. 95°C for 30 sec, annealing 55°C for 1 min, extension 72°C for 1min, final extension 72°C for 15min.

After completion, the PCR products were electrophoresed on 1% agarose gels, containing ethidium bromide (10mg ml), to ensure that a fragment of the correct size had been amplified. The amplification products were purified with K0701 GeneJET™ PCR Purification Kit (Thermo**).** Afterward, the samples become ready for sequencing in ABI Prism 3730XL DNA sequencer and analysis on GATC Company.

Phylogentic analysis and tree construction

Phylogentic data were obtained by aligning the nucleotides of different 16S rRNA retrieved from BLAST algorithm (www.ncbi.nlm.nih.gov/BLAST), using the CLUSTAL W program version 1.8 with standard parameters. The classifier is trained on the new phylogenetically consistent higher-order bacterial taxonomy (Ribosomal Database Project, RDP Classifier) proposed by Wang *et al.,* (2007).

Phylogenetic and molecular evolutionary analyses were conducted using Mega 6 program (Tamura *et al.,* 2013). All analyses were performed on a bootstrapped data set containing 100 replicates (generated by the program).

Nucleotide sequence ID

The nucleotide sequences of 18S rRNA gene of *Trichoderma asperellum* SRBP_ZSHSG1 have been deposited in GenBank and MycoBank under accession number**:** KP336489 and MB811455.

Secondary structure prediction and Restriction site analysis

The RNA secondary structure of the isolate SRBP_ZSHSG1 was predicted according to Brodsky *et al.,* (1995) using Genebee online software by the greedy method and the

restriction sites of the DNA of the strain was analyzed by NEB cutter Version 2.0 according to Vincze *et al.,* (2003).

Fermentation medium

The modified medium rice straw according to El-Gamal and Hamed (2003) was prepared and inoculated with *T. asperellum* SRBP_ZSHSG1 and incubated at 28:30 °C for 10 days. To prepare the biostraw is used in soil treatment. Cut rice straw and moistened with water until it reaches the humidity to 70%. 5 kg of rice straw was packaged in thermal bags, be added urea by 2%, 5% Soil and 5% molasses close the bags and sterilized. Then inoculated with 5% from the previous fermentation medium processing and incubated for 15 days. To obtain biostraw extract is used in soil treatment. Biostraw former outfitted soaked in a 5 liter of water for 24 hours and then taken filtrate.

Extraction of activated compounds

Flasks which containing fermentation medium were inoculated and incubated on a rotator incubator shaker at 180 rpm for 10 days at 28-30°C. Antibiotics were extracted from the culture filtrate. The culture filtrate was adjusted at pH 5.1 and extracted three times with 250 ml ethyl acetate/l. Aqueous layer was removed; solvent layer was washed three times with 35 ml of 5% sodium bicarbonate solution, followed by twice with water. The solvent layer was concentrated in vacuum till dryness and re-dissolved in ethyl acetate (Sawai *et al.,* 1980).

Biological evaluation of fungal isolates *in vitro*

Saprophytic fungal isolates were evaluated as antagonistic organisms *in vitro* against pathogenic fungi, the causal agent of root-rot and/or wilt diseases (*Fusarium solani, Fusarium oxysporum* and *Sclerotium rolfesii*) using C'zapek-Dox agar medium. Also the culture filtrate of *T. asperellum* SRBP_ZSHSG1 was evaluated using the same pathogenic fungi.

Preparation of saprophytic fungi inocula for antagonistic test was performed by removing disks 10 mm. in diameter from edge of expanding colonies grown on C'zapek-Dox agar. A disk was placed on the Petri dish containing C'zapek-Dox agar medium seeded with pathogenic fungus incubated at 25-28°C for 3-5 days and inhibition zones in Cm rounded each disk were measured. Three replicates were used for each fungus (Hamed *et al.,* 2012).

Determination of activated compounds by GC/MS

The extracted compounds were identified by (GC/MS) analysis of the crude extraction (Nawar 2008). GC/MS analysis was performed on a Varian gas chromatography interface to SSQ 3400 coupled to mass selective detector, the columns used was a DBS, 30M x 0.25Mm, 0.5 Mm film thickness injector and ion source temperature was 220 C^o, the ionization energy was set at 70 eV, and the volume injected was 088 µl at 270 °C, the oven temperature was programmed from 50 C^o for 32 minutes, isothermal, then heating by 10°C/ min. to150 ^oC, isothermal, then heating by 5 min to 270 °C, and isothermally for 3 min at 270 ^oC (Adams 1995).

Field experiment

Field experiment on Lupine was carried out at Sahl El-Hosainia Station, El-Sharkia Governorate, Egypt, during winter season. Biocstraw was plowed 15 days before planting at the rate of 300 kg fed $^{-1}$. Use the biostaw extract at a rate of 300 liters per Fadden.

Determination of cartenoides, chlorophyll and total phenol

Total cartenoides were determined spectrophotometrically (in diethyl ether) at the wavelength of 440 nm as mentioned by Beadle and Zscheili (1942). Total phenols were determined as the method of Folin ciocalten (Heda *et al.,* 2005). Total chlorophyll, mass determined in fresh leaves according to Saric *et al.,* (1976).

Statistical analysis

Analysis of variance (ANOVA) was carried out using Mstatc program. The Least Significant Difference (LSD) at P _0.05 and 0.01was applied to detect differences among treatments (Nawar 2008).

RESULTS AND DISCUSSION

Isolation of fungal isolates from Al-Hosainia locality

The results given in Table 1 showed that 16 fungal isolate belonging to 8 genera namely: *Alternaria, Aspergillus, Cladosporium, Curvularia, Fusarium, Penicillium, Sclerotium* and *Trichoderma* were obtained from the rhizosphere of the examined plants at Al-Hosainia locality.

Table. 1: Fungal species isolated from sugar beet rhizosphere at Al-Hosainia locality.

Fungal genera		Fungal species
1-	*Alternaria*	*Alternaria solani*
2-	*Aspergillus*	*Aspergillus niger* *A. flavus* *A. tamari* *A. terreus*
3-	*Chaetomium*	*Chaetomium globosum*
4-	*Cladosporium*	*Cladosporium cladosporioides* *Cladosporium herbarum*
5-	*Fusarium*	*Fusarium oxysporum* *Fusarium solani* *Fusarium semitectum*
6-	*Curvularia*	*Curvularia Boedjjn*
7-	*Sclerotium*	*Sclerotium rolfesii*
8-	*Trichoderma*	*Trichoderma asperellum* *T. Viride* *T. roseum*

Aspergillus was the most common genus (4 isolates), *Aspergillus niger, A. flavus, A. tamari, A. terreus* represented 25% of the total fungi produced. *Fusarium* (3 isolates) as *Trichoderma* isolates which represented by 3 isolates equivalent to 18.75% of the total fungi.

Identification and taxonomic classification of fungal isolate SRBP_ZSHSG1

Phenotypic identification of the isolated fungi

Fig. 1 present the morphological characters the isolated fungi.

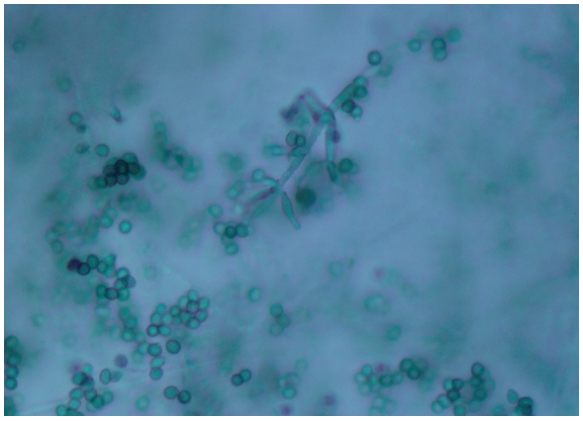

Fig. 1: The morphology of *Trichoderma asperellum* SRBP_ZSHSG1, showing mycelium branching and conidio-spores growing on Czapek-Dox agar medium 28 °C.

The primer specificity, Sequencing and phylogenetic analysis

The percentage of G+C is one of many general features used to characterize bacterial genomes. The G+C content of the genomic DNA was 55 mol% for SRBP_ZSHSG1 strain was obtained from the phylogenetic analysis. These results were in accordance with those by Storck (1966) and Nakase (1971) who mentioned the GC content of fungi ranges from 31.5 to 63%. This range is larger than it is for each class. The compositional diversity also decreases from classes and subclasses to genera and species.

The genomic DNA of SRBP_ZSHSG1 isolate was subjected to PCR using universal primers to amplify the ITS1 and ITS4 regions between the small and large nuclear rDNA, including the 18S rDNA. These primers amplified a DNA fragment of about 580 bp. These results were in agreement with Freeman, *et al.*, (2000) & Rasu, *et al.*, (2012) who found that these primers are specific for fungi and amplified a DNA fragment of about 560 bp using *Trichoderma asperellum* TTH 1.

The amplified PCR product was loaded in the 1% agarose gel. After electrophoresis, the gel with PCR fragment was excised under the UV transilluminator. Then they were purified with the using GeneJET™ PCR Purification Kit (Thermo) K0701. Finally, purified PCR product was checked on 1.5% agarose gel and then subjected to sequencing. DNA sequencing was performed on GATC Company by use ABI 3730xl DNA sequencer with forward and reverse primers.

The nucleotide sequence (580bp) of strain *Trichoderma asperellum* SRBP_ZSHSG1 was prepared by DNA STAR (DNASTAR. INC., Madison, Wis.) and blasting the sequence with the available Genbank resources using NCBI-BLAST search (www.ncbi.nlm.nih.gov/BLAST) to compare the SRBP_ZSHSG1 strain with those of member *Trichoderma* species strains. The results showed that the high sequence similarity species (100%) with *Trichoderma asperellum* strain.

Alignment, phylogenetic tree construction and GC%

The phylogenetic tree (Fig 2) showed that strains SRBP_ZSHSG1 is most closely related to *Trichoderma asperellum*. Therefore, it was proposed a name *Trichoderma asperellum* SRBP_ZSHSG1. The nucleotide sequence of 18S rRNA genes for SRBP_ZSHSG1strain has been deposited in GenBank under accession numbers: KP336489.

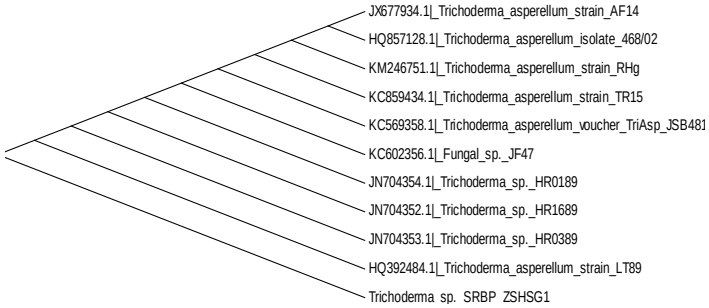

Fig 2 : Neighbor-joining phylogenetic representation of the strains and their closest NCBI (BLAST) relatives based on 18S rRNA gene sequences of *Trichoderma asperellum* SRBP_ZSHSG1and some known sequences of species.

Secondary structure prediction and Restriction site analysis

The RNA secondary structure was predicted for 18S rRNA of SRBP_ZSHSG1 (Fig 3). It showed that the free energy of structure is -150.3 kkal/mol, threshold energy is -4.0 with cluster factor, conserved factor 2 and compensated factor 4 and conservatively is 0.8. The prediction of restriction sites of SRBP_ZSHSG1 strain showed the restriction sites for various enzymes such as MseI, SmII, EcooO1091, BspCNI and BsoBI etc (Fig 4).

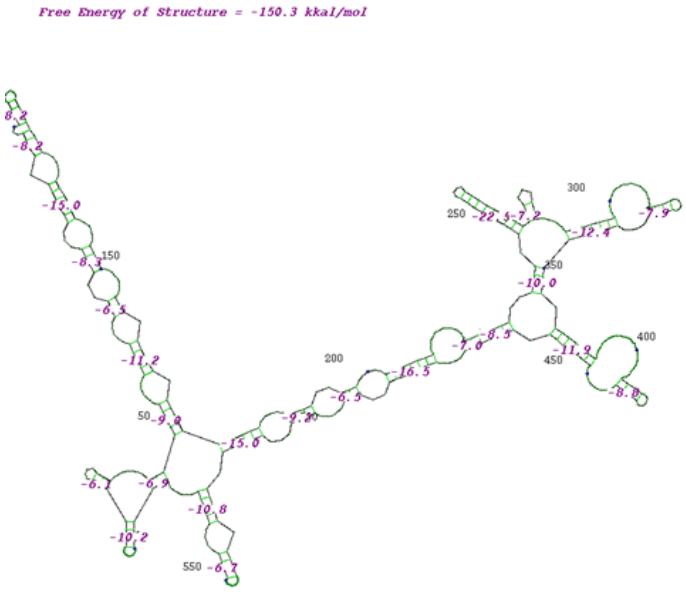

Fig. 3 : Secondary structure prediction of 16s rRNA of the strain *Trichoderma asperellum* SRBP_ZSHSG1was done using Genebee online software.

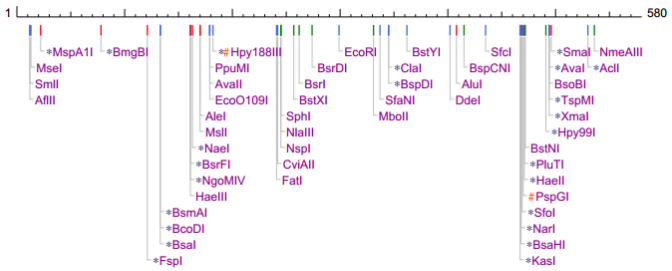

Fig 4 : Restriction sites of the strain *Trichoderma asperellum* SRBP_ZSHSG1 were predicted using NEB single cutter .

Antifungal activities

The antagonistic effect of saprophytic fungi test was carried out against 3 pathogen fungi (*Fusarium solani, Fusarium oxysporum and Sclerotium rolfesii).* The data given in Table 2 showed various degrees of antagonism against the fungal pathogens. About 3 fungal isolates were the most promising i.e. *T. asperellum* SRBP_ZSHSG1 followed by *T. rosium* and *Chaetomium globosum* were highly inhibitors to the tested pathogens. Also, the culture filtrate of *T. asperellum* SRBP_ZSHSG1 was the most inhibitor against the same pathogens.

Table 2: Biological activity of sapeophytic isolated fungi against pathogenic fungi.

Fungal isolates	Pathogenic fungi Diameter of inhibition zone in (cm)		
	F. solani	***F oxysporum***	***Sclerotium rolfesii***
Alternaria solani	3.10	3.3	2.20
Aspergillus niger	2.20	0.0	1.80
A. flavus	2.30	0.0	0.00
A. tamari	2.50	2.1	3.00
A. terreus	2.40	2.1	2.60
Chaetomium globosum	4.60	4.7	4.10
Cladosporium cladosporioides	1.55	1.4	2.65
Curvularia Boedjjn	0.00	0.0	2.95
Trichoderma asperellum SRBP_ZSHSG1	6.50	4.8	4.50
T. Viride	3.80	4.4	2.10
T. roseum	6.25	5.5	4.10
T. asperellum SRBP_ZSHSG1 (culture filtrate)	6.20	4.3	3.90

The extracts of the phylotypes *Trichoderma asperellum, Gibberella sp.,* and *F. oxysporum* displayed broad antifungal activity and inhibited the growth of all Colletotrichum's targets. *Trichoderma asperellum* is a widely distributed species in soil and has been considered an efficacious agent for biological control owing to its production of enzymes that hydrolyze cell walls of different microorganisms (Luiz, *et al*., 2012). Benítez *et al.,* (2004) and Mbarga *et al.,* (2012) showed that *T. asperellum* strains displayed an antagonistic effect against the phytopathogenic fungus *Pythium myriotylum.*

Other studies described the isolation of the secondary metabolites peptaibols and 2 trichotoxins from *T. asperellum*, suggesting that it is a rich source of new compounds Krause *et al.,* (2006) and Ren *et al.,* (2009) collected *T. asperellum* from sediment of the Antarctica and isolated 6 new peptaibols named asperelines, suggesting that this fungal species produces highly active compounds that can be used as a scaffold to the development of new drugs. The present study clearly indicates the potential of *T. asperellum* in inhibiting the mycelial growth of pathogen and production of cell wall degrading enzyme. Hence, *T. asperellum* would be a superior biocontrol agent for the control of plant pathogens.

The work has to be intensified to study other mechanisms involved in the inhibition of pathogen growth under in vitro conditions (Thilagavathi Rasu *et al*., 2012)

Separation and antimicrobial investigation of the extracts:

The ethyl acetate extract of culture filtrate of *Trichoderma asperellum* SRBP_ZSHSG1 was subjected to Gas chromatography/Mass spectroscopy (GC/MS).

Data in Table 3 show twenty four compounds were identified and classified into five categories as: benzenoid products, hydrocarbon compounds, volatile alcohol compounds, fatty acid and phthalic acid products.

Table 3: GC/MS of different products produced by *Trichoderma asperellum*

No	Name of hydrocarbon	Rt	Formula	%	M+
1	1,7-Octadiyne	2.15	C_8H_{10}	12.90	105
2	Phenol-3,4,5-tri- methoxy	3.4	$C_9H_{12}O_3$	0.22	178
3	Methyl–furancarbox- Aldehyde	5.35	$C_6H_6O_2$	0.12	110
4	2- Ethylacridine	6.66	$C_{14}H_9N$	1.91	207
5	β -Fenchol	8.38	$C_{10}H_{18}O$	3.96	154
6	Terpinene-1-ol	9.44	$C_{10}H_{18}O$	9.76	154
7	Phenol-2,4-dioctyl	9.52	$C_{22}H_{38}O$	10.08	318
8	Tetradecane	16.41	$C_{14}H_{30}$	3.04	198
9	9- Pentadecane	18.96	$C_{15}H_{32}$	3.37	212
10	10- 1-hexadecene	21.21	$C_{16}H_{32}$	1.04	224
11	11- Hexadecane	21.37	$C_{16}H_{34}$	2.75	226
12	Benzene (1-butyl- heptyl)	22.32	$C_{17}H_{28}$	1.07	232
13	Pentatriacontene	23.79	$C_{35}H_{72}$	1.08	492
14	Octadecan-3-ethyl-5(2ethylbutyl)	25.60	$C_{26}H_{52}$	2.3	366
15	Benzene (1-butyl- Nonyl)	26.74	$C_{18}H_{32}$	1.09	260
16	Phthalic acid butyl isohexyl ester	27.69	$C_{18}H_{26}O_4$	3.01	306
17	Nonadecane	27.96	$C_{19}H_{40}$	1.08	268
18	Benzene (1-methyl- dodecyl)	28.27	$C_{19}H_{32}$	1.27	260
19	Hexadecanoic acid methyl ester	28.63	$C_{17}H_{34}O_2$	3.95	270
20	Hexadecanoic acid ethyl ester	29.98	$C_{18}H_{36}O_2$	1.97	284
21	Heptadecanoic acid ethyl ester	31.35	$C_{18}H_{38}O_2$	0.09	298
22	9,12-Octadecadienoic acid (z,z) methyl ester	31.93	$C_{19}H_{38}O_2$	0.09	294
23	Phthalic acid-iso Hexylisopropyl ester	35.13	$C_{17}H_{22}O_4$	1.58	290
24	Phthalic acid dibutyl ester	39.97	$C_{16}H_{22}O_4$	4.69	278

Trichoderma asperellum SRBP_ZSHSG1 has been applied in soil, and *T. koningii* on cowpea leaves, as a biocontrol agent against *Rhizoctonia solani* in a greenhouse environment, (Latunde 1991 and Lartey *et al*,. 1994) and against wood degrading fungi (Canessa and Morrell 1996). *T.* species are recognized bio-pesticides mainly against *Rhizoctonia, Sclerotinia* and *Botrytis* (Lewis and Papavizas 1991).

In vitro studies, *Rhizoctonia solani, Pythium ultimum* and *Chalara alegans* were strongly inhibited by *Trichoderma viride, T. asperellum* SRBP_ZSHSG1, *T. pseudokoningii* and *T. koningii*, when both the pathogen and antagonist were grown in pairs in the

same agarized medium and when they were grown on separate media in a confined environment. These results indicated that the biocontrol efficacy of *Trichoderma* seems to perform not only at medium, but also at atmosphere level (Marchetti *et al.*, 1992).

Field experiment

Field experiment on Cowpea was carried out at Sahl El-Hosainia Station, El-Sharkia Governorate. Controlling of the most aggressive pathogenic fungi of cowpea root rot was carried out under field conditions. Percentage of pre-and post-emergence damping-off were assessed at 40, 60 and 90 days from sowing, respectively (Table 4). A complete randomized block design with three replicates was applied. Data represent in Table 5 showed that all treatments were able to reduce the linear growth of the pathogens in comparing with the control. Biostaw extract was the most effective bioagent and recorded highest reduction followed by biostaw.

Table 4: Effect of biostraw and biostaw extract on damping- off and seedling survivals of cowpea after 40, 60 and 90 days.

Treatment	Days damping off%								
	40			60			90		
	pre	Post	survival	pre	post	survival	pre	post	survival
Control	21	40	39	70	20	10	-	-	-
Biostraw	9	19	72	16	16	68	16	22	62
Biostaw extract	4	12	84	7	14	79	8	18	74

Table 5: Effect of biostraw and biostaw extract on growth parameters of cowpea plant 60 days after sowing

Treatment	Plant height (cm)	No of branches	No of leaves
Control	60	4	100
Biostraw	77	6	177
Biostaw extract	84	8	193
LSD	4.60	0.44	8.11

Fig. 5 and Table 6 indicate that treatment with biostaw extract or its combination with biostraw reduced damping off caused pathogens and increased healthy survival plants compared to untreated infected (control). Biostaw extract was the most effective and lowest percentage of disease incidence and high increase in healthy survival plants followed by biostraw only.

Fig. 5 : Field experiment on Cowpea was carried out at Sahl El-Hosainia Station, El-Sharkia Governorate. 1: biostaw extract, 2: control, 3: biostaw.

Table 6: Effect of biostraw and biostaw extract on yield of Cowpea.

Treatment	No of pods	No of seeds	Weight of 100 seeds
Control	-	-	-
Biostraw	14	24	31
Biostaw extract	16	30	36
LSD	0.81	0.14	0.177

Compost technology is a valuable tool already used to increase yields by farmers interested in sustainable agriculture. Compost contains a large varied microbial population of actinomycetes, bacteria, filamentous fungi and yeast, (Mcquilken *et al.*, 1994). There are several successful examples of biocontrol by application of compost to suppress a wide range of soil borne and foliar diseases. The assemblages of microorganisms present in composts are complex and dynamic and can be extremely effective in reducing disease.

Several mechanisms of action for this phenomenon have been proposed by (Hoitink *et al.*, 1993) including interspecific competition for nutrients, production of chemicals with antimicrobial activity, production of enzymes that destroy the cell walls of pathogens, and changes in the environmental conditions of the soil, which inhibit pathogen growth, were also included. As reported by Bohanomi (2010) disease control with compost has been attributed to four possible mechanisms 1-successful competition for nutrients by beneficial microorganisms 2-antibiotic production 3-successful predation against pathogens 4-activation of disease – resistant genes in plant.

Compost enriched soil showed suppression of plant diseases and the beneficial uses of composts can help growers by save money, reduce their use of pesticides and conserve natural resources solubilization and nitrogen fixation (Tartoura *et al*., 2005& Siddigui *et al*., 2008).

The fungal mycelia might have been inhibited not only by antibiosis but also why other antifungal metabolites such as siderophores, hydrogen ions and gaseous products including ethelyne , hydrogen cyanide and ammonia. Many investigations confirmed these results such as Handelsman *et al.*, (1990) and Alaa El-Dein (2013).

Cartenoides, chlorophyll and total phenol

Results in Table 7 reveled that total phenols in the plant tended to increase gradually with present biostaw extract gave highest value of Phenol, cartenoides and chlorophyll A and B compared with control. Plant phenolic compounds are secondary metabolites. The beneficial effects of those molecules are related to their antioxidant activity.

Table 7 Effect of biostaw extract with biostraw phenol and photosynthetic pigment content of Cowpea plants 60 days after sowing.

Treatment	Total phenol mg/100g/dry weight	Photosynthetic pigments mg/100 g fresh weight		
		Chl A	Chl B	Carotenoides
Control	356	1.1	0.72	0.19
Biostraw	428	1.54	1.09	0.27
Biostraw extract	515	1.48	1.01	0.31
LSD	40.26	0.05	0.06	0.01

They provide protection against pathogens (Sakr & El-Metwally 2009). Cartenoides and chlorophyll A and B are known to play a role in abiotic stresses these compounds are largely responsible for the red yellow and orange color of fruit and vegetables. In plants they have several functions ecologically they provide flowers and fruits distinct colors and seconts to attract animals that promote pollination and seed dispersal or that antagonize plant pathogen (Diretlo *et al*., 2007).

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