Plant Archives Volume 20 No. 2, 2020 pp. 8353-8362 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ROLE OF *TRICHODERMA* SPP. IN IMPROVING COMPOST PROPERTIES

Mona M. El-Shazly

Soil Fertility and Microbiology Department, Desert Research Centre, Cairo, Egypt.

Abstract

To increase the efficiency of composting process, selected fungal isolate *Trichoderma reesei* was used with powerful activities toward cellulase and xylanase enzymes production. Compost tea from produced compost was used as biocontrol agent for pathogenic fungi .15 fungal isolates were examined for production of Cellulase and xylnase enzymes, the highest isolate in enzymes production was identified using 18sRNA and subjected to quantitative determination of enzymes. HPLC analysis for total phenols and flavonoids in *T.reesei* extracts. Composting materials were prepared including plant and animal residues. After composting produced the produced compost wasanalyzed for NPK, Fe, Zn, Mn, C/N ratio was calculated and microbiological properties were determined. Compost tea prepared from traditional and biocompost analyzed for mineral components, microbial counts, phenol and flavonoids contents. Compost tea was used as biocontrol agent against some plant pathogenic fungi *Aletrnaria solani, fusarium oxysporum and Rhizoctonia solani.* Qualitative examination for production of cellulase and xylanase showed that 6 and 3 fungal isolates showed cellulase and xylnase, most active fungal isolates in enzymes production was identified as *Trichoderma reesei* which was subjected to quantitative determination for enzyme production being 1684 and 385U/L for cellulase and xylnase respectively. HPLC analysis for *T.reesei* methanol extract recorded presence of Kampeferol. Produced biocompost with *T. reesei* application recorded highest nitrogen content than traditional compost. Compost tea was prepared from the two composts and used as bioconrol for pathogenic fungi showed inhibition percentage rate of 41, 53, 30 and 92, 81, 74 for traditional and biocompost respectively against *F.oxysporum, A.solani and R.solani.* The HPLC chromatogram of methanolic extract of traditional and biocompost tea showed the presence of Phenantherine and kaempferol in traditional compost tea and recorded presence of Resorcinol, kaempferol and Quercetin in biocompost tea which illustrated the biocontrol efficiency of biocompost tea. It could be concluded the stimulating effect of *T.reseei* in improving compost efficiency, using biocompost tea as biocontrol for disease suppression.

Key words: Compost - Trichoderma - Biological control- compost tea

Introduction

Egyptian dessert soils are generally poor in their fertility, especially organic matter content. Utilization of desert soil requires addition of organic matter to improve its fertility. Organic matter is produced from the remains of living organisms (plant or animal) that returned to the soil and decomposed. Organic matter existing on the soil surface as raw plant residues helps to protect the soil from the effect of rainfall, wind and sun. Organic matter within the soil serves several functions to soil and plant. It is important for two main reasons: (i) as a "revolving nutrient fund"; and (ii) as an agent to improve soil structure, maintain tithe and minimize erosion. Organic matter releases nutrients in a plant-available form upon decomposition. In order to maintain this nutrient cycling system, (A. Bot and J. Benites 2005).

Compost is a rich source of organic matter which plays an important role in sustaining soil fertility and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physicochemical and biological properties of the soil. Compost application significantly improve the physical properties of studied soil such as bulk density hydraulic conductivity and moisture contents. Also, increase the available N, P and K in the cultivated soil, soil organic matter, Ca^{2+}, Mg^{2+}, K^{+} and P, while C: N ratio was narrowed. Hence, there is a general increase in nutrient supplying capacity of soils (Sarwar, *et al.*, 2010).

The use of biocompost of organic wastes is considered to be a promising alternative way to mineral

fertilizers as it reduces the amount of applied mineral fertilizers and at the same time improves the chemical and microbiological properties of desert under less polluted environment. From the economical point of view, such application reduces the agricultural costs, increase the yield of inoculated crops by providing them with an available nitrogen source and growth promoting substances [Abd El-Gawad, 2008].

Biofertilizers are supposed to be a safe alternative to chemical fertilizers to minimize the ecological disturbance. Biofertilizers are cost effective, eco-friendly, improve soil texture, pH and other properties of soil, produces plant growth promoting substances IAA amino acids, vitamins (Mukhopadhyay, 2006).

Composts contain an astonishing variety of microbes, many of which may be beneficial in controlling pathogens. Beneficial microbes help to control plant pathogens through either specific or general suppression. Fungi penetrate throughout the composting material, decomposing both chemically and mechanically organic matter fraction such as lignins and cellulose. Fungal hyphae physically stabilize the compost into small aggregates, providing the compost with improved aeration and drainage. Ecologically, fungi play a vital role in breakdown of dead plant materials. (Gaber and Heba 2005).

Compost is a mixture of decayed organic material decomposed by microorganisms in a warm, moist and aerobic environment that release nutrients into readily available forms for plant use. Among these microorganisms, fungilike *Trichoderma* spp. are important microbes that help in decomposition of organic material and are known as "compost fungal activator" (CFA).

Trichoderma spp. are widely known as a lignocellulosede composer, flamentous and have the ability to produce prolifc spores which can invade substrates quickly. Various studies have shown that composting of lignocellulosic material spreinoculated with potential *Trichoderma* spp. can reduce the time of biodegradation. *T. harzianum* was used as inoculant to enhance composting of rice straw and weeds (Tengerdy and Szakacs, 2003, Mohammad and Alam, 2012 and Parkash and Saikia, 2015).

The major barrier to the use of compost as a substrate or biocontrol agent has been the variation in physical and chemical characteristics and disease suppression levels across and within compost types, sources and batches. Aerated and non-aerated compost teas, both products of compost, have also been shown to suppress soil-borne diseases, including damping-off and root rots like *Pythium ultimum, Rhizoctonia solani* (Scheuerell and Mahaffee 2006; Dionne´ *et al.*, 2012), wilts produced by *Fusarium oxysporum and Verticillium dahliae* (Alfano *et al.*, 2011), among many others. Most of the papers published on the control of pathogens by means of compost tea have studied pathogens from the aerial part of plants and the number of trials that use non-aerated compost tea being higher (Dia´nez *et al.*, 2006). Therefore, the aim of this work was to study the role of Trichoderma in improving compost properties.

Materials and Methods

Soil Sampling and Isolation of Compost Fungal Activator

Ten grams of soil were weighed out and topped up to 100 mL with sterilized distilled water and shaken with the orbital shaker for 30 min at 210 rpm. After that, 1 mL of the solution was added to 9 mL water for the first (10^{-1}) dilution. The serial dilutions of 10^{-3} and 10^{-5} were used for Colony Forming Unit (CFU) measurements. Approximately 1 mL of soil solution was pipetted out and seeded into each Petri dish followed by pouring 9 mL of sterilized Trichoderma Selective Medium (TSM) [0.20 g of $MgSO_4 7H_2O$, 0.90 g of K_2HPO_4, 0.15 g of KCl, 1.0 g of NH_4NO_3, 3.0 g of glucose, 0.15 g of Rose Bengal, 20.0 g of agar (Difco, USA) and 1000 mL of distilled water] as reported by Elad and Chet, (1983). Each Petri dish was swirled manually before being allowed to solidify and then incubated at 28±2°C for 7 days.

The fungal isolates obtained were subcultured on sterile culture plates containing Potato Dextrose Agar (PDA) and Czapek media until pure culture of each isolate was obtained. Each isolate was code labeled and subcultured regularly to maintain viability.

Screening of fungal isolates for Celluolase and xylanase activities

The fungal isolates were subjected to qualitative screening for their cellulolytic and xylanolytic activities using the modified agar diffusion test method (Whitaker *et al.*, 2002). Ten microliters (10 µl) of spore suspension of each isolate was dropped onto 6 mm diameter of sterile paper disc cut out of Whatman No. 1 filter paper.

The inoculated paper discs were dried at room temperature in a lamina flow chamber and put onto the center of special medium plates in which xylan or carboxylmethyl cellulose had beenincorporated. Cellulase activity was evaluated by using acellulose-agar medium containing 1% Carboxyl methyl cellulose or beech wood xylan as a substrate for celluolase and xylanase respectively. Plates were spot inoculated with spore

suspension of pure culture and incubated at 28°C. After 5 days, 10 ml of 1% Congo red staining solution was added to the plates for 15 min. Then, the Congo red staining solution was discarded and 10 ml of NaOH (1 N) was added to the plates for 15 min. Then it was removed and the staining of the plates was measured by noticing the formation of yellow zones around the fungal spore-inoculated disc. The diameter of the clear zone of decolorization around each colony was measured.

Isolate with wide clear zones was selected for further work. Pure cultures of the selected fungal isolate was regularly sub-cultured onto fresh sterile Potato Dextrose Agar (PDA) and kept in refrigerator at 4°C.

Enzyme assay

Enzyme assay Cellulase and xylanase activities were assayed by measuring the amount of reducing sugars released from cellulose and xylan, respectively, using dinitro salicylic acid (DNS) assay as described by (Baldrian and Gabriel, 2003). and (Bailey *et al*., 1992). Briefly, 0.5 mL of culture supernatant was added to 1 mL of 0.05 M citrate buffer of pH 4.8. To this mixture, 0.5 mL 1% w/v carboxy methyl cellulose (CMC) was added as a substrate for cellulose assay and 0.5 mL 1% w/v beech wood xylan was added as a substrate for xylanase assay. All the samples were incubated at 50°C for 30 min. To this, 2 mL of DNS reagent was added, heated in water bath at 90°C for 10 min and cooled immediately. Development of color was visible and the absorbance was measured in a spectrophotometer at 540 nm.

Reducing sugar concentration was determined using glucose standard for cellulase activity and xylose standard for xylanase activity. Both cellulase and xylanase activities were reported as U/L. One unit of activity was expressed as the amount of enzyme required to release 1 mol of reducing sugar/min under assay conditions.

Identification of selected fungal isolate

Most active isolate was selected for further study and identified using 18S rRNA at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Maintenance of *Trichoderma reesei*

The microorganism was cultured and stored on potato dextrose agar (PDA) medium at 4°C and was subcultured in regular intervals. Glycerol stocks of 80% were prepared and stored at -20°C for future use. Sporulation inoculum was prepared by adding two loops full of spores from PDA plates into sabouraud dextrose broth and incubated at 30°C in ashaker for three days and stationary for next three days. Sporulation occurs and this is used as inoculum for all the systems.

Compost Preparation

Preparation of substrate: Farm wastes including plant wastes and fallen leaves were dried, chopped to speed up decomposition by increasing the surface area available for microbial action and providing better aeration. Woody material should be passed through a grinder. All existed agricultural wastes were subjected to analysis before composting for C/N ratio which was as follow: plant wastes contained N being 0.6% and C/N ratio being 44%, fallen leaves contained N being 0.29% and C/N ratio being 38%.

Composting Method: Farm wastes were spread in layer alternates with wet animal dung which is spread over this layer in rate 1 animal dung to 5 farm wastes. Adjust humidity content of compost to 60%. *Trichoderma reesei* was added to accelerate decomposition process. After that, it covered with plastic sheet and anaerobic decomposition commences. In four weeks, the mass becomes reduced and the heap flattens. The plastic cover is removed and the entire mass is turned. Aerobic decomposition commences at this stage. Water is sprinkled to keep the material moist. The compost is ready for use after two to three months Day and Shaw, (2001).

Compost can be enriched by

-Addition of Nitrogen and phosphore makes the compost more balanced and supplies nutrient to microorganisms for their multiplication and faster decomposition. The addition of P also reduces N losses. (Salem, 2006).

Application of Calcium ammonium nitrate (33.3% N) and calcium super phosphate (15.5 % P_2O_5) were added in concentrations of 20 Kg/ton and 5 Kg/ton assources of nitrogen and phosphorous, respectively. Calcium carbonate was added in concentration of 20 Kg/ton to neutralize the pH of compost.

Compost analysis

Microbiological Determinations

Microbial analysis of obtained compost were determined. total microbial counts as described by Nautiyal (1999) and For counting and growing fungi using Potato Dextrose Agar medium (PDA) as reported by Elad and Freeman, (2002).

Chemical Analysis of compost

Electrical conductivity (EC) was also measured every week. Ten grams of each compost sample was added to 100 mL of distilled water (1:10 v/v), placed in a shaker

for 30 min and then kept for 24 h. Salinity was determined using an Electrical Conductivity Meter and expressed as dS/m.

The organic carbon was determined based on the oxidation of organic matter with potassium dichromate and sulphuric acid. The excess potassium dichromate was titrated. Total nitrogen was determined by the micro-kjeldahl method according to Page *et al*., (1984). Fe, Mn, Zn, Mg, K, Na, Ca and NO_3 were also determined.

Compost tea

Compost tea preparation

Compost tea was prepared by brewing compost and water at a ratio of 1:5 w/v (compost:water) supplemented with 2% molasses to stimulate microbial growth and continuous aeration for 24 hr (Naidu *et al*., 2010). After that, compost tea was filtrated and diluted two times for further application. Chemical and biological properties of the compost tea were measured.

Chemical properties of compost tea. The pH level and electrical conductivity (EC) were measured using an electrical conductivity and pH meter. Total nitrogen was estimated by the micro-kjeldahl method according to Page *et al*., (1984).

Microbial population density of composttea. Microbial population density of composttea was analyzed. Compost tea samples were taken after 24 h brewing cycle for each aerated composttea. Total microbial counts determination on nutrient agar (NA, nutrient broth), Total fungi on potato dextrose agar (PDA) and rose bengal agar (RBA) based PDA and added 67 mg/l rose bengal (Baggerman, 1981).

After incubation at 25°C for 24 h, population densities were reported as colony forming unit (cfu)/ml from each media.

HPLC analysis of phenolic compounds in *Trichoderma reesei* extract and compost tea

The high-performance liquid chromatography (HPLC) analysis was performed according to Biswas *et al*., (2013) . The system thermo (ultimate 3000) consisted of: pump, automatic sample injector and associated Dell-compatible computer program. Adiode array detector DAD-3000 was used. The Thermo-hypersil reversed phase C18 column 2.5×30 cm was operated at 25°C. Mobile phase consisted of distilled water (solvent A) and methanol (solvent B.). The UV absorption spectra of the standards as well as the samples were recorded in the range of 230-400 nm. Samples and standards solutions as well as the mobile phase were degassed and filtered through 0.45um membrane filter (Millipore). Identification of the compounds was done by companion of their retention time and UV absorption spectrum with those of the standards.

Antagonistic activity of compost tea against pathogenic fungi (Lab experiment)

Pathogenic fungi used

Three fungal pathogens were used to assess the suppressiveness of the prepared compost tea. *Fusarium oxysporum, Alternaria solani a*nd *Rhizctonia solani* were maintained on potato dextrose agar (PDA; at 25°C).

Effect of compost tea on mycelial growth of pathogenic fungi (in vitro)

The assay aimed to determine the in vitro inhibitor effect of compost tea on mycelial growth selected pathogenic fungi Compost tea obtained as described above was filtered. After filtration, filtrate was centrifuged at 10,000 rev/min for 10 min. PDA was sterilized and cooled to 45 C. pathogenic fungi were inoculated and pour plates containing fungi, then after solidification make well in each plate using corkborrer and add 1 ml of compost tea extract (Dia´nez *et al.,* 2006). Controls consisted of adding distilled water instead of compost teas. incubated 6–7 days in the dark at 25 C After the incubation period, clear zone diameters was calculated compared to controls. All experiments were conducted according to a completely randomized design with five repetitions.

Effect of compost tea on mycelial growth of pathogenic fungi (in vivo)

Greenhouse experiments

Tomato seedlings of highly susceptible cultivar to Fusarium wilt disease (*cv.* super strain B) were provided from Agricultural Research Centre, Giza.

Tomato seedlings were cultivated in plastic pots (25 cm diameter, 5.0 kg of soil) were filled with sterilized soil artificially infested with *F. oxysporum* isolate.

Compost tea was applied as soil drench except control after 7 days of seedling cultivation and every week with three concentrations (100, 75 and 50%) from traditional compost tea and biocompost tea . Healthy tomato seedlings (40day-old,) were transplanted in plastic pots at the rate of 1 seedlings/pot, following three replicates for each treatment along with two check treatment as control soil infested with pathogen and control with non-infected sterilized soil.

Plant height, number of fruits per plant, fruit weight per plant (kg), fruit weight (g), fresh weight and dry weight were noted at the end of greenhouse experiment.

Disease severity

Wilt severity were estimated at 21 days after transplanting using scale 1-6, according to Silva and Bettiol (2005). They were as follows: 1 = no symptom, 2 = plant showed yellowing leaves and wilting 1- 20%, 3 = plant showed yellowing leaves and wilting 21- 40%, 4 = plant showed yellowing leaves and wilting 41- 60%, 5 = plant showed yellowing leaves and wilting 61- 80% and 6 = plant showed yellowing leaves and wilting 81-100% or die. Virulent group was categorized according to Disease severity index (DSI) as non-pathogenic (DSI =1), low (DSI ≤ 3.50), moderate (DSI > 3.50 - 4.50) and high (DSI > 4.50). The wilt disease incidence was carried out using a visual 1- 6 scales according to (Silva and Bettiol, 2005).

Statistical analysis

Least significant difference (LSD) was used to estimate the differences between means at $p < 0.05$.

Results and Discussion

1-Isolation, Purification and Screening Of Cellulolytic and Xylanolytic Fungi

Selection procedure following the method of Zhang, (2014), the selected strains producing high cellulose and xylanase levels were members of the *Trichoderma* groups since they are fast growing and highly sporulating fungi.

In this study, cellulase and xylanase-producing fungal isolates were obtained. Cellulolytic and xylanolytic fungi were presumptively identified by the qualitative plate assay method. In this study, a total of 15 fungal isolates were obtained from rhizosphere soil were screened for cellulose degradation. Six of these isolates were positive when cellulose was used as a sole carbon source, whereas three isolates were able to degrade xylan as the sole source of carbon. Cellulase and xylanase activities were indicated by the halo zones around the colonies. The diameters of halo zones were measured and the results were illustrated in table 1. Of all only one isolate recorded positive both xylanase and cellulase activities; however, this isolate was chosen for further work table 1.

Table 1: Qualitative determination of cellulose and xylanase.

No.	Inhibition zone diameter(cm)		No	Inhibition zone diameter(cm)	
	Cellulase	Xylanase		Cellulase	Xylanase
1	-	-	9	-	-
2	-	-	10	-	-
3	2.3	-	11	3.8	3.7
4	1.9	-	12	-	-
5	-	-	13	-	-
6	4.6	3.2	14	4.2	-
7	-	-	15	2.6	3.1
8	-	-			

Table 2: HPLC analysis for Phenol and flavonoids in T, reesei methanol extract.

No.	Peak name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount ppm
1	Quercetin	3.583	6.163	70.136		19.72	78.16
2	kaempferol	3.790	18.768	114.930		32.31	233.15

HPLC analysis for phenolic content of *T.reesei*

The HPLC chromatogram for methanol extract of *T.reesei* showed the presence of quercetin and kaempferol as presented in Fig. 1 and table 2. Quercetin is known as aglycones but also as glycosides. It is reported to display anti-histamine, anti-cancer as also antiinflammatory activities which mostly follow its antioxidant traits. (Wach *et al.*, 2007). Among natural poly-phenolics, kaempferol, is a flavonol found in many edible plants and is reported to possess potent pharmacological and nutraceutical activities. Kaempferol displays several pharmacological properties, among them antimicrobial, anti-inflammatory, antioxidant, antitumor, cardioprotective, neuroprotective and antidiabetic activities and is being applied in cancer chemotherapy. The antioxidant properties are known to be responsible for these health benefits (Imran, 2019, Aboody and Mickymaray 2020). The present investigation showed the presence of kaempferol content in the methanol extract (233.4 mg/gm) and quercetin (78.15 mg/gm).

Identification of most active fungal isolate in enzyme production

Most active isolate in cellulase and xylanase production was selected for further study and identified using 18S rRNA as *Trichoderma reesei* according to the molecular and morphological identification unit and stored in the microbiology laboratory culture collection unit of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

Quantitative determination of cellulase and xylanase enzymes in Trichoderma medium

Table 3: Quantitative determination of cellulose and xylanase enzyme produced by *T.reesei.*

	Cellulase U/L	Xylanase U/L
Trichoderma reesei	1684	385

Production of cellulase and xylanase from *T. reesei* was reported in table 3. During the *T. reesei* cultivation in lignocellulosic medium, cellulose and xylan act as inducers for cellulase and

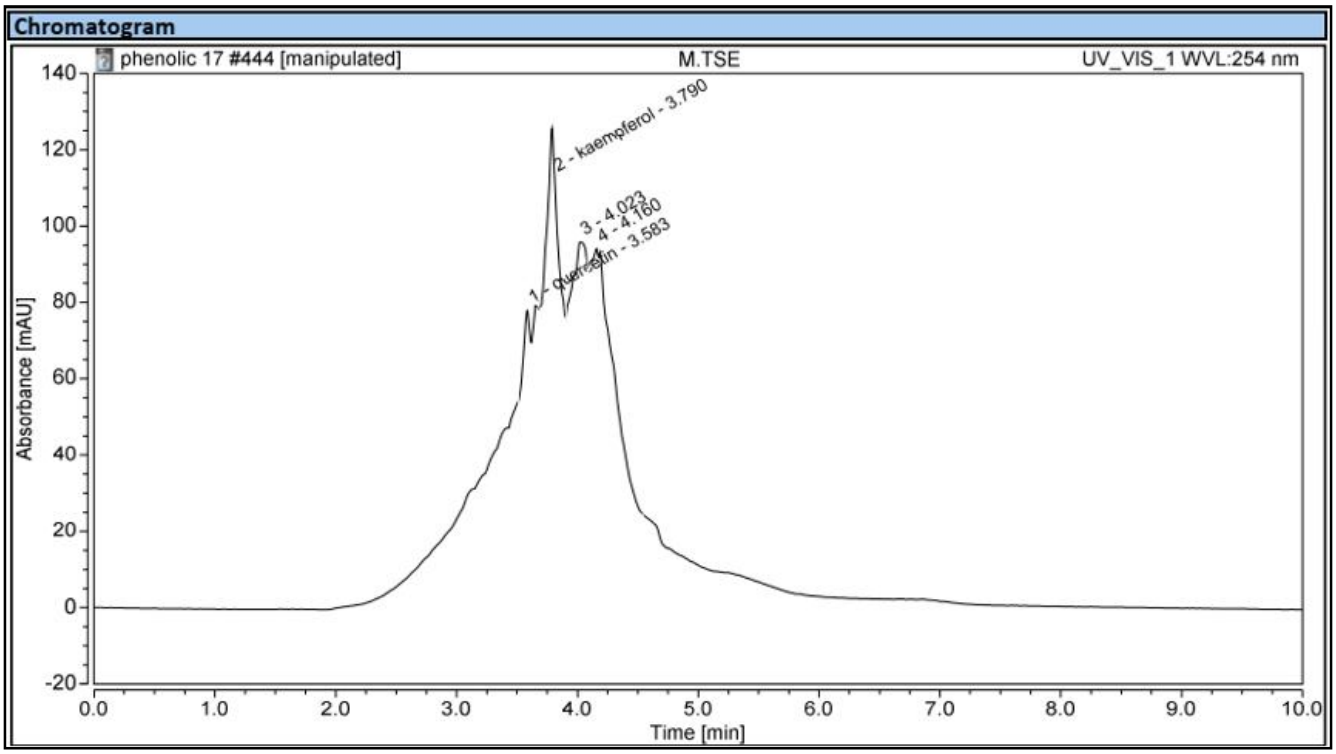


Fig. 1: HPLC analysis for Phenol and flavonoids in *T. reesei* methanol extract.

xylanase activities, respectively. *T. reesei* yielded a significant level of cellulase, 1684 U/L and xylanase, 385 U/L activities. This results in accordance with Jampala *et al.,* 2017.

Table 4: ChemicalAnalysis of compost.

Parameter	Traditional compost	Biocompost
PH	7.9	7.5
E.C ds/m	4.36	4.19
Total N%	0.7	1.1
Total C%	16.45	14.6
C/N ratio	23.5	13.27
P ppm	190	243
K ppm	850	1015
Mgppm	128	140
Na ppm	375	349
Ca ppm	225	229
Fe ppm	867	871
Mn ppm	13.7	15.1
Zn ppm	24.29	27.8

Table 5: Microbiological analysis of compost.

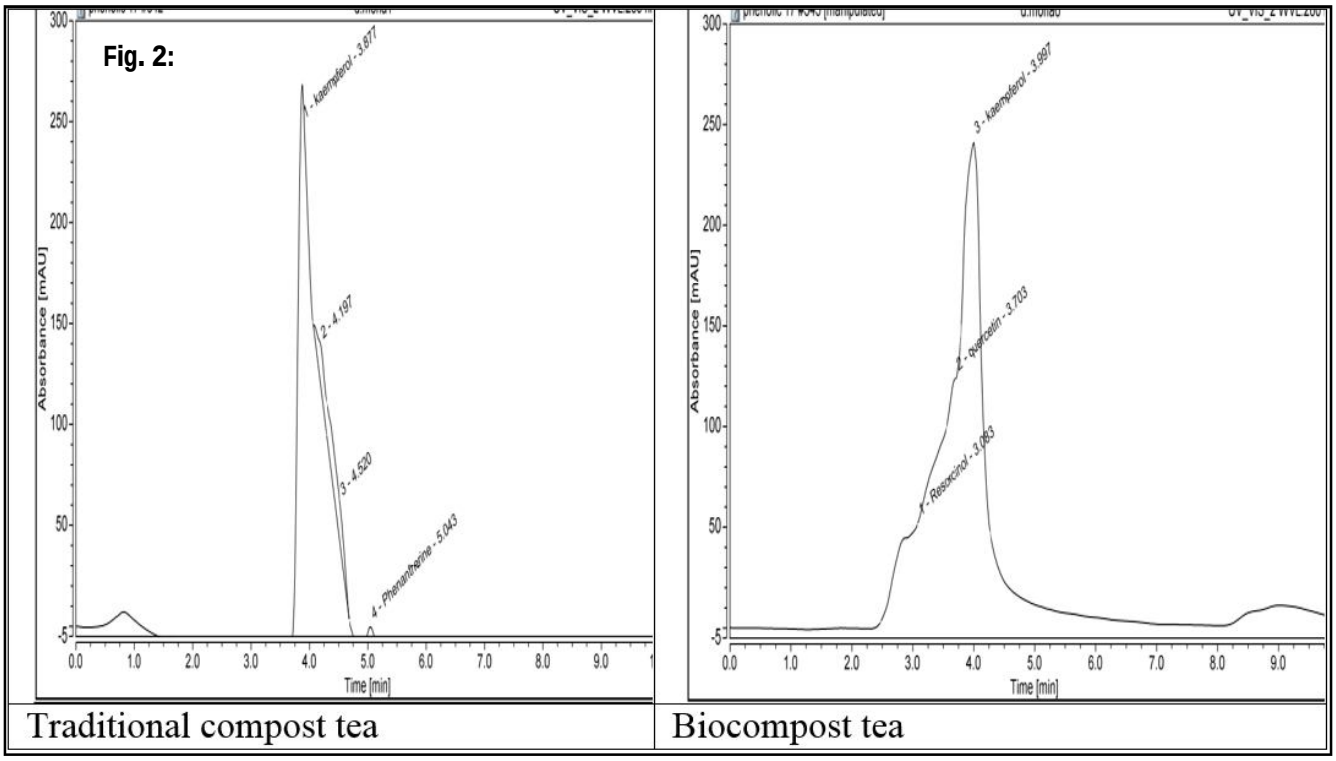
Parameter	Traditional compost	Bio-compost
Total microbial counts	210×10^5	$>300\times10^5$
Azotobacter densities	26×10^3	85×10^3
Phosphate dissolving fungi(PDB)	9×10^2	27×10^2
Cellulose decomposers	15	69
Fungi Counts	28	51
CO_2 evolution (mg CO_2 / 100 g dry soil/24 hr)	23	34

Compost Analysis

Two type of composting process were done, traditional compost without trichoderma and biocompost with trichoderma application .The rapid decomposition can be detected by a pleasant odour, by the heat produced (visible in the form of water vapour given off during the turning of the pile), by the growth of white fungi on the decomposing organic material, by a reduction of volume and by the materials changing colour to dark brown. As near completion, the temperature drops and finally little or no heat is produced. The compost is then ready to use. table 4 and 5 showed the chemical and microbiological analysis of resulted two types of compost.

It is clear that all macro, micronutrients, heavy metals and organic matter are in the accepted ranges. Both N content and C/N ratio are very close to the reported values by Abd El-Gawad, 2008.

Microbial examination of obtained compost reveled the increase in numbers of beneficial microorganisms like azotobacters, phosphate dissolving bacteria, aerobiccellulose decomposers and total microbial counts and highest value for CO_2 evolution as indicator for improving microbial respiration and activity These results are in compatible with Indira *et al.,* (2004) and Salem, (2006). By this time, the temperature of the compost has



also stabilized at about 35°C. As a result of this inoculation, the N content of compost can be increased by up to 2 percent. In addition to improving N content and the availability of other plant nutrients, these additions help to reduce the composting time considerably.

Table 6: Chemical and biological properties of the compost tea.

Parameter	TCT	BCT
pH	7.9	7.4
E.C(ds/m)	4.41	4.28
N ppm	1016	1250
P ppm	86.7	107.5
K ppm	722	867
Microbiological properties(cfu/ gm)		
Total microbial counts	82×10^6	190×10^6
Total fungi	21×10^2	33×10^2
Total actinomycetes	14×10^2	27×10^2
Total coliform	Nil	Nil

TCT: Traditional biocompost tea, BCT: Bio-compost tea

Chemical and microbial analysis of compost tea

Chemical and microbiological analysis of prepared compost tea (ratio 1:4 w:v) are shown in table 6. Compost tea produced from previously made compost, chemical and microbiological analysis showed the priority of biocompost tea in most studied properties compared with traditional biocompost tea this result in accordance with (Ibrahim *et al.*, 2019).

HPLC analysis for traditional and biocompost tea

The HPLC chromatogram of methanolic extract of traditional and biocompost tea showed the presence of Phenantherine and kaempferol in compost tea of traditional compost as presented in Fig. 2 and table 7. Biocompost tea showed the presence of Resorcinol, kaempferol and Quercetin.

In vitro effect of compost teas on mycelial growth of *F.oxysporum, A.Solani and R.solani*

Table 7: HPLC analysis for traditional and biocompost tea.

No.	Peak name	Retention Time min	Area mAU*min	Height mAU	Relative Area %	Relative Height %	Amount Ppm
Traditional Compost Tea							
1	Phenantherine	5.043	2.907	15.614	2.13	4.64	36.83
2	kaempferol	3.877	123.8	280.7	90.86	83.4	4578
Biocompost Tea							
1	Resorcinol	3.083	18.028	48.7	16.4	12.5	415.1
2	Quercetin	3.703	16.007	111.7	14.5	28.6	
3	Kaempferol	3.997	76.027	229.7	69.1	58.9	2810

Two type of compost tea (Traditional compost TCT, Biocompost BCT) were used in vitro experiment at concentration (100%) to explain effectivnesss in suppression of different pathogenic fungi. Obtained results showed visible inhibition of mycelial growth of tested pathogens compared to the controls. Biocompost tea proved highly suppressive against mycelial growth of tested pathogenic fungi table 8.

Inhibition was significantly greater (almost twofold) with application of BCT. Application of BCT resulted in mycelial inhibition that ranged from 86 to 90%.

The results of this study support the importance of the biotic components of compost extracts in suppressing disease caused by soil or foliar plant pathogens (Alfano *et al.,* 2011; Pane *et al.,* 2014).

Greenhouse experiments

Table 8: Inhibitory effect of compost tea treatments on mycelial growth of *F.oxysporum, A.solani and R.solani* Inhibition rate %.

	F.oxysporum	*A.solani*	*R.solani*
TCT	41	53	30
BCT	92	81	74

Table 9: Effect of treatments on disease severity of wilt in Tomato plants (cv. super strain) under greenhouse conditions.

Treatments	Disease severity%
TCT 100%	38.3
TCT 75%	49.2
TCT 50%	66.8
BCT 100	12.4
BCT75%	26.1
BCT 50%	43.5
Control (infected)	100
Control (uninfected)	0
L.S.D at 5%	0.157

Table 10: Effect of compost tea (TCT, BCT) on growth and yield parameters of tomato plantsunder greenhouse conditions.

Treatments	Plant height Cm	Fresh weight Gm/p	Dry weight Gm/p	No. of fruit/ Plant	Weight of fruit /Plant gm
TCT 100%	71	34.9	21.6	7.8	29.1
TCT 75%	52	31	19.4	4.9	27
TCT 50%	49	23.9	15.2	4.64	25.3
BCT 100	85	38.2	22.9	8.4	36.1
BCT75%	73	35.8	22.1	8.1	34.8
BCT 50%	68	33.2	21.3	6.5	32.1
Control (infected)	33	19.4	11.9	2.1	18.1
Control (uninfected)	43	21.5	13.6	4.2	24.6
L.S.D at 5%	1.79	0.623	0.237	0.084	0.308

Three concentrations of compost tea (TCT and BCT) amended with *Trichoderma reesei* were tested against wilt disease of tomato caused by *Fusarium oxysporum* under greenhouse conditions.

Effect of treatments on disease severity of wilt disease of tomato plants under greenhouse conditions

Results in table 9 indicated that three tested concentrations of compost tea significantly reduced wilt disease of tomato plants which decreased disease severity between 12.4 and 66.8%. The most effective was BCT at concentration 100% and reached their minimum records at the highest concentration of compost tea by 12.4% compared with other treatments. This results in agreement with (Ibrahim and El-Fiki, 2019).

Effect of compost tea (TCT, BCT) on growth and yield parameters of tomato plants under greenhouse conditions

All of the tested compost tea concentrations significantly increased the tested growth parameters i.e. Plant height (cm), Fresh and dry weight (gm), no. of fruits /plant, fruit weight/plant compared with infected control treatment as shown in table 10. Biocompost tea at all concentrations recorded highest values for all studied parameters compared with Traditional compost tea. Biocompost tea proved to be more effective in enhancing tomato growth. This results in compatible with (Ibrahim and El-Fiki, 2019).

Conclusion

The end outcome of this study is the benefit of being able to convert waste materials into a valuable by product with addition of higly efficient strain of *Trichoderma reseei* which characterized by high enzymatic activity for cellulase and xylanase. Obtained compost provides a high source of macronutrients (N, P and K), improves soil acidity, electrical conductivity and microbial community which considered key factor in composting process. The composting materials are easy to degrade and eco-friendly in nature and the resultant compost can be categorized under nutrient-enriched biocompost. Also, compost tea application in vitro for biocontrolling pathogenic fungi recorded inhibition rate reached 92% for *F.oxysporum*. We recommended with using *Trichoderma reseei* in making compost and using produced compost tea in biological control for pathogenic fungi.

References

Abd El-Gawad, A.M. (2008). Employment of Bio-

organic Agriculture Technology for *Zea mays* Cultivation in Some Desert Soils. *Research Journal of Agriculture and Biological Sciences*, **4(5):** 553-565.

Aboody, M.S.A. and S. Mickymaray (2020). Anti-Fungal Efficacy and Mechanisms of Flavonoids. *Antibiotics*, **9(2):** 45.

Alfano, G., G. Lustrato, G. Lima, D. Vitullo and G. Ranalli (2011). Characterization of composted olive mill wastes to predict potential plant disease suppressiveness. *Biol. Control*, **58:** 199–207.

Baggerman, W.I. (1981). A modified rose bengal medium for the enumeration of yeasts and moulds from foods. *European journal of applied microbiology and biotechnology*, **12(4):** 242-247.

Bailey, M.J., P. Biely and K. Poutanen (1992). Interlaboratory testing for methods of assay of xylanase activity. *J. Biotechnol.*, **23:** 257-270.

Baldrian P. and J. Gabriel (2003). Lignocellulose degradation by *Pleurotusostreatus* in the presence of cadmium. *FEMS Microb. Lett.*, **220:** 235-240.

Biswas, N., Pauline, Balac, Sai, Narlakkanti, E. Haque and M. Hassan (2013). Identification of phenolic Compounds in Processed Cranberries By HPLC Method. *J. Nutr. Food Sci.*, **3:** 1.

Bot, A. and J. Benites (2005). *The importance of soil organic matter: Key to drought-resistant soil and sustained food production* (No. 80). Food & Agriculture Org.

Day, M. and K. Shaw (2001). Biological, chemical and physical processes of composting. Compost utilization in horticultural cropping systems, 17-50.

Dianez, F., M. Santos, A. Boix, M. De Cara, I. Trillas, M. Aviles and J. Tello (2006). Grape marc compost tea suppressiveness to plant pathogenic fungi: role of siderophores. *Compost Sci. Util.*, **14:** 48–53.

Dionne, A., R.J. Tweddell, H. Antoun and T.J. Avis (2012). Effect of non-aerated compost teas on damping-off pathogens of tomato. *Canadian Journal of Plant Pathology*, **34(1):** 51-57.

Elad, Y. and I. Chet (1983). Improved selective media for isolation of *Trichoderma* spp. or *Fusarium* spp. *Phytoparasitica*, **11(1):** 55.

Elad, Y. and S. Freeman (2002). Biological control of fungal plant pathogens. In: The Mycota XI: Agricultural Applications. Kempken, ed. Springer-Verlag, Berlin.

El-Sersawy, N.M., Bouthaina F. Abd El-Ghany, K.W. Khalil and S.Y. Awadalla (1998). New Approaches for managing newly reclaimed soil with utilization of natural resources. *Egypt. J. Appl. Sci.*, **13(4):** 307-334.

Gaber, Z. and A. Heba (2005). Bio-production of compost with low pH and high soluble phosphorus from sugar cane bagasse enriched with rock phosphate. *World Journal of Microbiology and Biotechnology*, **21(5):** 747-752.

Ibrahim, H.A., R.M. Khater and R.H. Hegab (2019). Evaluate the effect of compost tea and some chelated micronutrients forms on black cumin productivity. *SN Applied Sciences*, **1(1):** 28.

Ibrahim, H.A. and I.A.I. El-Fiki (2019). Study on the effect of yeast in compost tea efficiency in controlling chocolate leaf spot disease in broad bean (Vicia faba). *Organic Agriculture*, **9(2):** 175-188.

Imran, M., B. Salehi, J. Sharifi-Rad, T. Aslam Gondal, F. Saeed, A. Imran and S.G. Guerreiro (2019). Kaempferol: A key emphasis to its anticancer potential. *Molecules*, **24(12):** 2277.

Indira, S., N.G. Singh and A.C. Singh (2004). Studies on the nutrient content and quality assessment of compost enriched with biofertilizer. *J. of interacadimicia*, **8(4):** 571-574.

Jampala, P., S. Tadikamalla, M. Preethi, S. Ramanujam and K.B. Uppuluri (2017). Concurrent production of cellulase and xylanase from Trichoderma reesei NCIM 1186: enhancement of production by desirability-based multi-objective method. *3 Biotech*, **7(1):** 14.

Mohammad, N., M.Z. Alam, N.A. Kabbashi and A. Ahsan (2012). Effective composting of oil palm industrial waste by filamentous fungi: A review. *Resources, Conservation and Recycling*, **58:** 69-78.

Mukhopadhyay, S.N. (2006). Ecofriendly products through process biotechnology in the provision of biotechnology economy-Recent advances. Technorama, A. Supplement to IEI News, Marc.

Naidu, Y., S. Meon, J. Kadir and Y. Siddiqui (2010). Microbial starter for the enhancement of biological activity of compost tea. *Int. J. Agric. Biol.*, **12(1):** 51-56.

Nautiyal, C.S. (1999). An effeicient microbiological growth mediun for screening phosphate solubilizing microorganisms. *FEMS Microbiology Letters*, **170:** 265-270.

Page, A.L., R.H. Miller and D.R. Keeney (1984). Methods of Soil Analysis. 2nd Edn., Amercen Society of Agronomy, Madison, WI., USA.

Pane, C., A.M. Palese, G. Celano and M. Zaccardelli (2014). Effects of compost tea treatments on productivity of lettuce and kohlrabi systems under organic cropping management. *Italian Journal of Agronomy*, **9(3):** 153-156.

Parkash, V. and A.J. Saikia (2015). Production and multiplication of native compost fungal activator by using different substrates and its influence on growth and development of Capsicum chinensis Jacq."Bhut Jolokia". *Biotechnology research international*, 2015.

Russell, E.W. (1989). "Soil Conditions and Plant Growth" ELBS edition of eleventh edition, 1988, Reprinted 1989.

Salem, A.M.O. (2006). New biotechniques for the production of the manure from organic wastes and their application on desert soil. Ph.D Thesis, Faculty of science, Banha University.

Sarwar, G., H. Schmeisky, A. Tahir, Y. Iftikhar and N. Sabah (2010). Application of green compost for improvement in soil chemical properties and fertility status. *Journal of Animal & Plant Sciences,* **20(4):** 258 – 260.

Scheuerell, S.J. and W.F. Mahaffee (2006). Variability associated with suppression of Gray Mold (*Botrytis inerea*) on geranium by foliar applications of nonaerated and aerated compost teas. *Plant Dis.*, **90:** 1201–1208.

Silva, J.C.D. and W. Bettiol (2005). Potential of non-pathogenic Fusarium oxysporum isolates for control of Fusarium wilt of tomato. *Fitopatologia Brasileira,* **30(4):** 409-412.

Tasia, W. and R. Melliawati (2017). Cellulase and xylanase production from three isolates of indigenous endophytic fungi. In *IOP Conference Series: Earth and Environmental Science,* **101(1):** p. 012035). IOP Publishing.

Teather, R.M. and P.J. Wood (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl. Environ. Microbiol.*, **43:** 777-780.

Tengerdy, R.P. and G. Szakacs (2003). Bioconversion of lignocellulose in solid substrate fermentation. *Biochemical Engineering Journal*, **13(2-3):** 169-179.

Wach, A., K. Pyrzyn´ska and M. Biesag (2007). Quercetin content in some food and herbal samples. *Food Chemistry,* **100:** 699–704.

Whitaker, J.R., A.G.J. Voragen and D.W.S. Wong (2002). Handbook of Food Enzymology, Routledge, USA. pp. 879–886.

Zhang, L., X. Wang, Z. Ruan, Y. Liu, X. Niu, Z. Yue, Z. Li, W. Liao and Y. Liu (2014). Fungal cellulase/xylanase production and correspondinghydrolysis using pretreated corn stover as substrates. *Appl. Biochem. Biotechnol.*, **172(2):** 1045–1054.