

## In Vitro Suppressive Effect of Agriculture Residues and Municipal Solid Wastes Compost Tea on some Phytopathogenic Fungi

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### Abstract

Aerated Compost Tea (ACT) prepared from four types of compost; Agricultural Residues (AR), Agricultural Residues enriched with Olive Pomace (AR+OP), Municipal Solid Wastes (MSW) and Municipal Solid Wastes enriched with OP (MSW+OP) in combination with bioagents have been analytically characterized and were tested for their antagonistic activity in vitro against *Fusarium oxysporum* and *Rhizoctonia solani* pathogens in comparison with the commercial chemical fungicides (Rhizolex-T). Assessment was carried out in terms of percent mycelial growth inhibition as well as Scanning Electron Microscopy (SEM) were used to explain the mode of action in the biological control of the pathogens under study. From the results all compost teas showed high levels of nutrients and microbial biomass content. The mixed treatment of compost tea gave the highest percent inhibition compared with individually treatments. AR compost tea+ Olive Pomace (OP)+ Microorganisms (MO) were significantly superior to the rest treatments showed 63.52% and 44.07% percent inhibition, same trend was detected with MSW+ Olive Pomace (OP)+ Microorganisms (MO) showing 57.41% and 43.89% against *Rhizoctonia solani* and *Fusarium oxysporum* respectively. SEM of tested pathogen mycelium removed from the confrontation zone showed aberrant morphology such as shrinkage, curling, mycelium asymmetry, partial distortions and lysis of fungal mycelium. In conclusion, these results provide evidence that the compost extract has the potential to become a good candidate for biological control as potential alternatives to the application of synthetic fungicides, and as plant promoters in crop production, for attaining environmental sustainability for farming and food safety.

**Keywords** : Compost, Compost tea, Bioagents, Olive pomace, *R. solani*, Rhizolex-T, Microorganisms (MO).

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## 1. Introduction:

Compost tea are fermented extracts of composted materials carried out on liquid phase in commercial or rudimental brewers, for a few days or up to two weeks with or without active aeration (Scheuerell & Mahaffee, 2002; Litterick et al., 2004; Ingham, 2005). Several studies have shown that compost and its liquid preparations actively enhances soil fertility, sustains productivity and provides efficient control of weed seeds and several plant pathogens (Joshi et al., 2009; Zaccardelli et al., 2011). In this regard, compost teas are viewed as potential alternatives to the use of the common synthetic fungicides in response to the increasing needs of environmental sustainability for farming and food safety (El-Morsi & Abdel-Monaim, 2015). Compost prepared from different feedstock varies considerably in chemical, physical and biotic composition and consequently vary in ability to suppress soil borne diseases (Castaño et al., 2011). So the amendments of specific microorganisms to compost are sometimes necessary, since the potential of compost to suppress plant disease is a highly variable phenomenon (Yang et al., 2011; Zhao et al., 2011; Huang et al., 2011). Yogen et al. (2006) and Khalil & El-Maghrabia (2010) found that Compost and/or compost tea fortified with bio-control agents (bio-compost) when applied as soil amendments were able to reduce pathogens propagates density and protect plants from soil borne pathogens. Larger quantities of waste products associated with olive oil production are being disposed in the environment (olive leaves, olive pomace and olive mill waste water). In fact (Ergu et al., 2008) has proven that this Olive Pomace (OP) waste may potentially acts as good sources of plant nutrients and can be used as a soil conditioner/fertilizers amendments and proposed as one of the most suitable methods to restore soil fertility and resolve the problem of their disposal (Abu-Zreig & Al-Widyan, 2002; Bonanomi et al., 2006). Also, the plant polyphenols can be used as natural plant protection agent exhibiting antimicrobial activity to control infestations of bacteria (Baydar et al., 2006) and fungi (Bruno and Sparapano, 2007). Based on these assumptions, the principle objective of the proposed study is to evaluate in vitro the bio-potential of two types of compost tea from different feedstock and enriched with OP and biocontrol agents as single treatments and/or in combination to suppress fungal diseases caused by *Rhizoctonia solani* and *Fusarium oxysporum*, as well as Scanning Electron Microscope (SEM) to evaluate antagonistic effect against fungal pathogens. Teas are characterized for physical, chemical, microbiological and phytotoxicity.

## 2. Materials & Methods:

### 2.1. Microbial Inoculum:-

#### 2.1.1. Phytopathogenic fungi:-

Two lentil (*Lens culinaris*, Medik.) Pathogens; *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. lentis were provided by legume and forage diseases research department, Plant Pathology Research Institut, ARC, Giza, Egypt. Pathogenic fungi were isolated from naturally infested lentil stem showing damping – off and wilt disease. The fungus were regularly sub-cultured and maintained on potato dextrose agar (PDA) medium in a refrigerator at  $5 \pm 1^\circ\text{C}$  until use.

### 2.1.2. Bioagents:-

Bacterial strains (*Pseudomonas fluorescens* (IFO.2034), *Serratia marcescens* (WW4) and *Paenibacillus polymyxa* (local isolate of rhizobacteria) inoculants) and fungal strains (*Trichoderma harzianum* and *Trichoderma viride*) were supplied by Microbiology Dept., Soils, Water and Environment Research Institute, Agricultural Research Center (ARC), Giza, Egypt. *Pseudomonas* was grown on king's medium (Atlas, 1995), *Serratia sp.* was grown on peptone glycerol (Difco Manual, 1984), *Bacillus polymyxa* was grown on nutrient broth medium (Difco Manual, 1984) and *Trichoderma sp.* grown on *Trichoderma* selective (TSM) broth medium (Askew and Laing 1993) was developed by (El-Hassan, 2004). Cultures were incubated at 28°C until early log phase reached  $10^9$  viable cell  $\text{ml}^{-1}$  and were mixed in equal proportion to prepare 1 liter of concentrate containing  $10^9$  cell  $\text{ml}^{-1}$ .

### 2.2. Initial Compost and Compost tea preparation:-

Two types of compost under study; Agricultural Residues (AR) was provided from Ismaillia Agricultural Research Station, Ismaillia Governorate, Egypt while the other from Municipal Solid Wastes (MSW) was provided from Qatameia, Cairo, Egypt composting and recycling complex, Delta Biotec. Both types were enriched with olive pomace (10 %); the Raw Olive Pomace (ROP) was air dried at 30°C for 24 hours then ground to 0.5 mm by using ball mill and sieved to reduce the materials to fine particles. The mixtures are moistened to the appropriate degree (50-60%), and then both types were leaved for 21 days incubation period with frequent turning every week and following the humidity. At the end of the period of incubation, a complete analysis of the physical, chemical and microbiological characters was done. The main traits of composts and enriched compost with olive pomace are shown in Table (1).

Aerated compost tea was prepared from matured compost before and after enrichment with Olive Pomace (OP). The compost tea were prepared according to (Abdel-Wahab, 2008; Naidu et al., 2010). After elapsing of incubation period and before application, fulvic acid is added (25ml), the liquid mixture was filtered through cheese clothes and stored at 4°C; they were taken out 30 minutes before use (Znaidi, 2002). The bioagents mixtures were added to the freshly prepared compost tea according to (Abdel-Wahab, 2008) (compost tea act as a liquid carrier). The microbial enriched compost tea was maintained up to 3 days before application.

### 2.3. Analysis of compost teas:-

The chemical and microbiological analytical characters as well as maturity and stability index of compost teas were recorded at the end of the fermentation period; physical characters were detected according to (Jimenez & Garcia, 1989). Chemical properties were determined according to (Page et al., 1982). Extinction coefficient ( $E_4/E_6$ ) ratio was measured according to (Page et al., 1982), where  $E_4$  and  $E_6$  are the optical densities at 465 and 665 nm wavelengths, respectively.

Total phenolics were determined by the method of (Singleton & Rossi, 1965). Total count of mesophilic bacteria, fungi, and actinomycetes were determined using nutrient agar, Martin's media, and Jensen media, respectively and the plate count techniques method according to (Page et al., 1982). Dehydrogenase activity was assayed according to the (Casida et al., 1964). Phytotoxicity test was assayed using cress seeds (*Lepidium staviium* L.) to evaluate compost maturity and stability according to (Pare et al., 1997).

#### 2.4. In vitro test of antifungal activity of compost extract:-

The antifungal effect of the aqueous olive pomace extract, bioagent mixture, commercial fungicides and different types of compost teas on the linear growth of the tested fungi was studied in vitro by a poisoned food technique (Agarwal et al., 2001).

**Table (1): Main characteristics of solid composts used to produce compost teas by aerated fermentation.**

Property	AR	AR+OP	MSW	MSW+OP
Water holding capacity (%)	150.7	120	143.2	130
pH (1:10 extract)	7.1	7.7	8.10	8.3
EC (dS/m)	3.5	5.04	7.4	6.9
Organic carbon (%)	18.72	19.8	17.95	18.3
Organic matter (%)	32.27	34.1	30.95	31.5
Total- N (%)	1.5	1.6	0.8	0.9
C/N ratio	12.6	12.36	22.58	20.98
Available-P (mgKg <sup>-1</sup> )	356.8	315.4	296.97	282.6
Available-K (mgKg <sup>-1</sup> )	647.1	748	667.7	759.9
Available Fe (mgKg <sup>-1</sup> )	175.7	181.07	278.7	363.67
Available Mn (mgKg <sup>-1</sup> )	32.5	37.9	69.9	71.6
Available Cu (mgKg <sup>-1</sup> )	3.73	5.0	32.0	46.53
Available Zn (mgKg <sup>-1</sup> )	31.37	33.77	103.87	121.53
CEC (c mol/Kg)	117.8	120	106.8	113.7
Total Phenol content (%)	0.4	0.50	0.70	0.6
Total count of Bacteria (cfu/g)	3.8×10 <sup>8</sup>	5×10 <sup>8</sup>	2.7×10 <sup>8</sup>	4.7×10 <sup>8</sup>
Total count of Fungi(cfu/g)	6×10 <sup>6</sup>	8.1×10 <sup>6</sup>	5.8×10 <sup>6</sup>	6.3×10 <sup>6</sup>
Total count of Actinomycetes (cfu/g)	2.5×10 <sup>6</sup>	4×10 <sup>6</sup>	3×10 <sup>6</sup>	4×10 <sup>6</sup>
E.coli count	Nil	Nil	Nil	Nil
Salmonella & Shigella	Nil	Nil	Nil	Nil
Dehydrogenase activity (µgTPF <sup>+</sup> /g)	120.8	140.1	119.7	129

\*Tri-Phenyl-Formazan.

In case of OP, 10 gram was dissolved in 100 ml water, and then were filtered and mixed with molted sterile potato dextrose agar medium (PDA) to obtain the proposed concentration of 10 %

(v/v). Regarding fungicide; Rhizolex-T was incorporated into sterilized PDA medium after cooling to obtain final concentrations of 1, 5, 10 and 25 ppm. The medium was then poured into petri-dishes, while in case of bioagents mixture and compost tea; 10ml was incorporated into sterilized PDA medium after cooling. The medium was then poured into petri-dishes (with three replicates for each treatment), and untreated PDA medium was used as control. After solidification of the medium each plate was inoculated centrally with a mycelial disc (5mm diameter) taken from the outer margins of actively growing mycelium from PDA cultures of each isolate by sterile cork borer. Plates were incubated at  $28 \pm 2^{\circ}\text{C}$  and colony diameters were measured after 5 days (Mahmoud, 2000). When the untreated control had just covered the plate Percentage of inhibition was calculated using the following formula (Perveen & bokhari, 2012).

$$\text{Percentage of growth inhibition} = (C - T) / C \times 100$$

Where, C = average of three replicates of hyphal growth (cm) of test fungus in control plate and T = average of three replicates of hyphal growth (cm) of the same test fungus in plates treated with the tested material.

### 2.5. Evaluation of morphological changes via Scanning Electron Microscopy (SEM):-

Blocks of the investigated fungal isolate were prepared for SEM at The Regional Center for Mycology and Biotechnology, Al-Azhar Univ.. Fixation and dehydration procedures were performed using the programmable LEICA EM TP tissue processor model (A-1170), Specimens were then gold-coated (nearly 50 nm thickness) using an SPI Module TMSputter Coater and then examined using the high-vacuum mode of a JEOL JSM-5500LV Scanning Electron Microscope. Energy-dispersive X-ray spectroscopy (EDX) (Elad et al., 1983).

### 2.6. Statistical analysis:-

The obtained data were subjected to an analysis of variance (ANOVA) according to (Gomez & Gomez, 1984).

## 3. Result:-

### 3.1. Physico-chemical and microbiological analysis of compost tea:-

#### 3.1.1. Physico-chemical determinants:-

Chemical characteristics are shown in **Tables (2)**, after brewing period, the pH of four types recorded **6.92**, **7.41**, **7.12** and **8.05** for AR, AR+OP, MSW and MSW+OP, while in case of EC, it recorded **2.50** ds/m, **3.30** ds/m, **6.62** ds/m and **6.33** ds/m for AR, AR+OP, MSW and MSW+OP respectively.

Concerning nutrients and micronutrients composition; including organic carbon (OC), organic matter (OM), nitrogen (N), phosphorus (P), potassium (K) and micronutrients: iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) were significantly higher in olive pomace-enriched

compost tea (CT) as compared to compost tea alone, except for phosphorus, olive pomace-enriched CT exhibit lower values than compost teas alone.

Regarding  $E_4/E_6$  ratio, four types of compost teas exhibit low values; they recorded 2.46nm, 2.49nm, 3.67nm and 3.21nm for AR, AR+OP, MSW and MSW+OP respectively.

### 3.1.2. Microbiological assays:-

Microbial populations in the produced Aerated Compost Tea (ACT) are shown in **table (2)**, Culturable microbial population densities were significantly different from the initial compost materials illustrated in **table (1)**. They were predominantly bacteria, the average of their densities increased in the four types of compost teas after five days of brewing. The numbers of total aerobic bacteria were the highest among other microbial groups, such number is well appropriate for offering the treated plants with enough microbial coverage for pathogen suppression. Highest population density of culturable bacteria recorded  $10 \times 10^8$  cfu/ml in both mixture of AR compost tea and AR+OP compost tea. However, fungal population densities were lower than those of bacteria and increased in all compost teas after five days of incubation. The highest population density of fungi was recorded  $9 \times 10^6$  cfu/ml in AR compost tea and  $8.5 \times 10^6$  cfu/ml in AR+OP compost tea of incubation. On the other hand, actinomycetes were the lowest group in all treatments. The Dehydrogenase activity (DHA-ase) was a very reliable indicator of global metabolic activity of the organisms inhabiting the compost teas. Data presented in **table (2)** shows that four types of compost tea recorded high value of dehydrogenase activities; they recorded 133.6, 125, 131.8 and 130  $\mu$ g TPF/100ml/24hr for AR, AR+OP, MSW and MSW+OP respectively.

Germination Index (GI) is the best way to test the phytotoxicity of compost to plant growth. They are widely used to test for salinity, soil pathogens, toxic substances (such as phenolic compounds, high ammonia concentration and heavy metals) and some chemical properties of compost which could be the major potential reasons of phytotoxicity. Data presented in **table (2)** shows that four types of compost tea recorded high Seed Germination (SG) and Root Growth (RG) values, their Germination Index (GI) values were > 80 %.

**Table (2): physico-chemical and microbiological traits of the prepared compost teas.**

Parameters	AR compost tea	AR+OP compost tea	MSW compost tea	MSW+OP compost tea
<b>pH</b>	6.92±0.14c	7.41±0.29 b	7.12±0.05bc	8.05±0.13 a
<b>EC (dSm<sup>-1</sup> at 25<sup>0</sup>C)</b>	2.50±0.20 c	3.30±0.40 b	6.62±0.58 a	6.33±0.30 a
<b>Organic carbon (%)</b>	5.3±0.16 b	7.0±0.46 a	4.7±0.26 b	5.3±0.79 b
<b>Total- N (ppm)</b>	80.14±0.71 d	87±3.00 c	103.2±3.37 b	139±2.00 a
<b>Organic matter (%)</b>	9.14±1.04 b	12.0±0.76 a	8.10±0.44 b	9.14±0.38 b
<b>Available-P (ppm)</b>	58.30±1.61 a	57.0±3.00 a	47.03±1.79 b	34.1±1.32 c
<b>Available-K (ppm)</b>	253.6±3.14 d	460±13.23 a	281.8±6.01 c	391±7.65 b
<b>Extractable- Fe (ppm)</b>	12.9±0.10 b	15.0±2.00 b	17.5±0.56 a	18.3±1.47 a

Extractable- Mn (ppm)	3.0±0.26 c	10.2±0.82 ab	9.6±0.40 b	12.0±1.73 a
Extractable- Cu (ppm)	3.5±0.46 c	4.0±0.50 c	8.6±0.95 b	15.0±1.25 a
Extractable- Zn (ppm)	3.94±0.20 c	4.5±0.50 c	9.2±0.72 b	12.0±1.80 a
E <sub>4</sub> /E <sub>6</sub> ratio	2.46±0.10 c	2.49±0.06 c	3.67±0.12 a	3.21±0.25 b
Total count of Bacteria (cfu ml <sup>-1</sup> )	10×10 <sup>8</sup> ±1.00 a	10×10 <sup>8</sup> ±0.05 a	9×10 <sup>8</sup> ±0.14 a	7×10 <sup>8</sup> ±0.05 a
Total count of Fungi (cfu ml <sup>-1</sup> )	9×10 <sup>6</sup> ±0.09 a	8.5×10 <sup>6</sup> ±0.05 a	8×10 <sup>6</sup> ±0.04 a	7×10 <sup>6</sup> ±0.05 a
Total count of Actinomycetes (cfu ml <sup>-1</sup> )	4×10 <sup>6</sup> ±0.18 a	4×10 <sup>6</sup> ±0.05 a	5×10 <sup>6</sup> ±0.19 a	4×10 <sup>6</sup> ±0.13 a
Dehydrogenase activity (mg TPF <sup>**</sup> /ml/24hr)	133.6±2.33 a	125±2.00 b	131.8±2.03 a	130±2.00 a
Germination test of cress seeds	93.4±2.19 a	92±2.65 ab	87±2.65 c	89±1.00 bc

\*\*Tri-Phenyl-Formazan. \*\*\*values within row followed by the same letter are not significantly different according to Duncan (1995).

### 3.2. In vitro suppressiveness of phytopathogens:-

In vitro test were done to evaluate the efficacy of some botanical biocides (Aerated Compost Tea (ACT) prepared from four different types of compost, olive pomace extract, mixture of bioagents and their combination), compared with the standard synthetic fungicide (Rhizolex-T) against two soil-borne lentil crop pathogens (*Rhizoctonia solani* and *Fusarium oxysporum*).

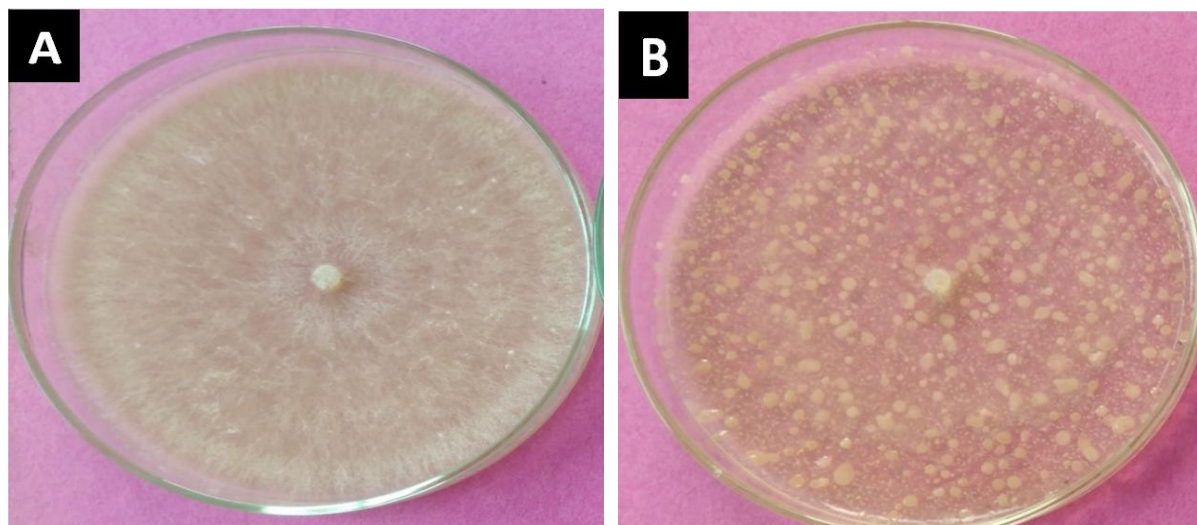
Table (3): in vitro growth reduction (%) of *Rhizoctonia solani* and *Fusarium oxysporum* in response to compost teas, bioagents mixture, OP and their combinations..

Treatment	Mycelial growth percentage inhibition (%)	
	<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i>
Olive pomace	46.67±2.23b	55.00±1.47a
Bioagents mixture	35.74±5.01c	28.70±4.66c
AR (CT)	41.67±2.78bc	25.22±0.30c
MSW (CT)	33.70±3.35c	30.37±0.85c
AR+MO	61.11±8.33a	30.19±1.16 c
MSW+MO	34.26±1.61c	39.26±3.16b
AR+OP	62.96±12.53a	30.56±3.34c
MSW+OP	38.89±4.81bc	41.48±4.02b
AR+OP+MO	63.52±4.53a	44.07±4.46b
MSW+OP+MO	57.41±4.24a	43.89±2.78b
Control	00.00d	00.00d
<b>Fungicides</b>		
1 ppm	83.70 ± 5.01b	5.80 ± 0.14d
5 ppm	100.00± 0.00a	12.01 ±1.43c
10 ppm	100.00± 0.00a	17.81 ± 2.35b
25 ppm	100.00± 0.00a	37.47 ± 1.90a

\*each value represents the mean of three replicates. Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test (p=0.01) (1995).

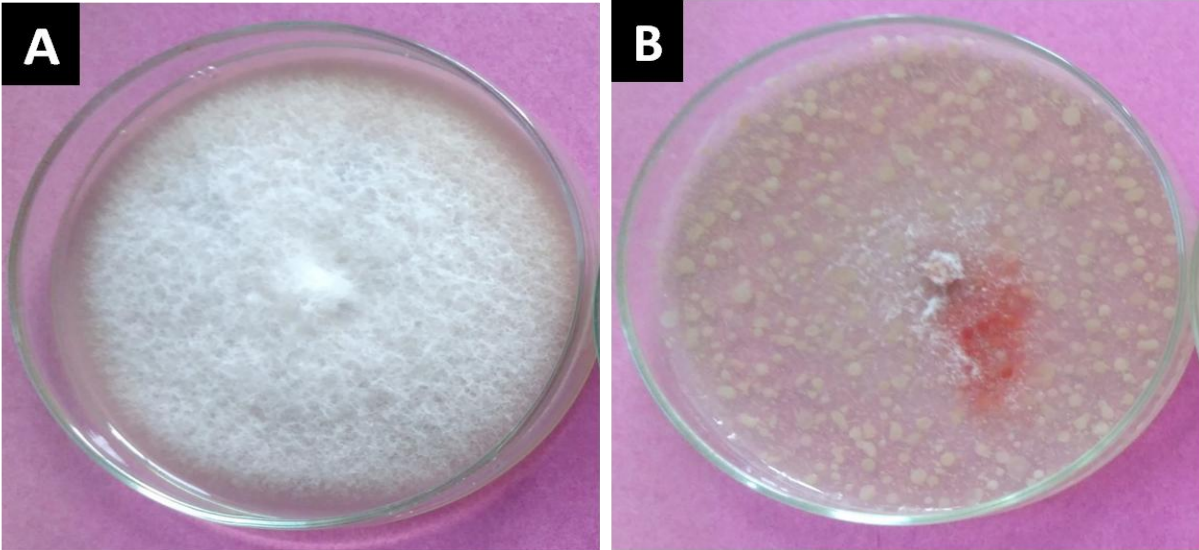
Treatment with compost tea, as a biological control approach, is a potential method to reduce the growth of pathogenic fungi. Data in **table (3)** indicate that all treatments in this study produced suppressive effect against the two soil borne pathogens. In fact, they inhibited *R. solani* mycelia growth more than *F. oxysporum*. Enrichment of compost with OP and microorganisms were the most effective treatments recorded 63.52% and 57.41% for *R. solani* while recorded 44.07% and 43.89% for *F. oxysporum* in case of AR+OP+MO and MSW+OP+MO respectively.

Regarding fungicides, four different concentrations of Rhizolex-T (1, 5, 10 and 25 ppm) were tested, **table (3)** reveal that the reduction in the growth of both tested fungi were correlated with increasing the commercial antifungal concentration (Rhizolex-T) in the medium. Data also indicate that both tested fungi varied in their sensitivity against the fungicides used. *Rhizoctonia solani* showed more positive response than *Fusarium oxysporum*, complete growth inhibition was recorded for *Rhizoctonia solani* at 5, 10 and 25 ppm, while in case of *Fusarium oxysporum* the growth inhibition increase from (5.83 % to 37.52 %) with increasing the concentration of Rhizolex-T from (1 to 25 ppm).



**Photo 1: Effect of AR compost tea plus OP and bioagents on linear growth of *R. solani*. 1 (A) control; 1 (B) AR+OP+MO.**

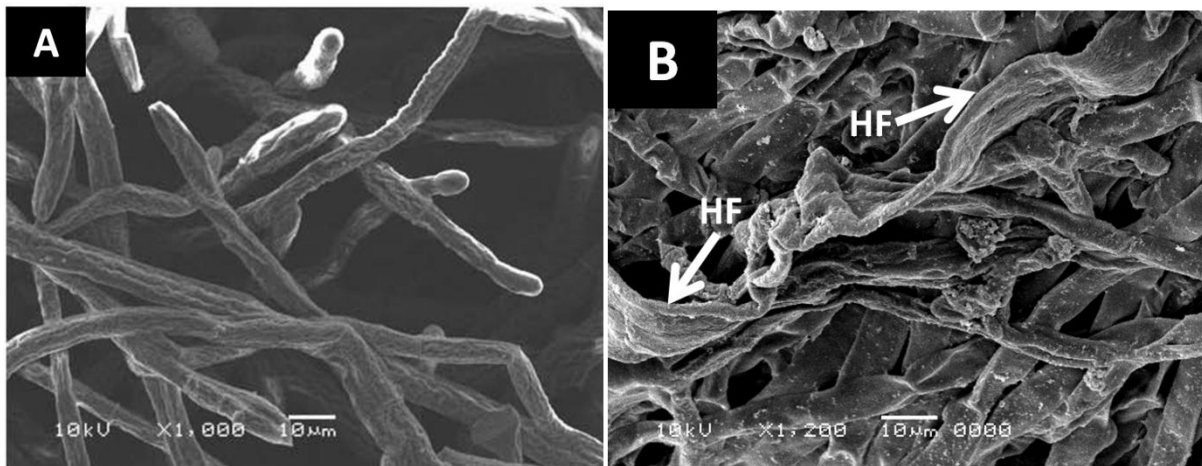




**Photo 2: Effect of AR compost tea plus OP and bioagents on linear growth of *F. oxysporum*.**  
2 (A) control; 2(B) AR+OP+Mo.

### 3.3. SEM effects of Compost tea on phytopathogens:-

The use of electron microscopy facilitated the study of the mode of antagonistic action. Mycelial samples from the interaction region of the highest affected treatment. SEM clearly demonstrated changes in the morphology of both pathogens in media containing the treatment (AR compost tea+OP+MO) extract. SEM observations of untreated (control) mycelia of *R. solani* are illustrated in Photo3(A). Showing long, even, and round hyphae with typical tapered apices and a smooth surface.



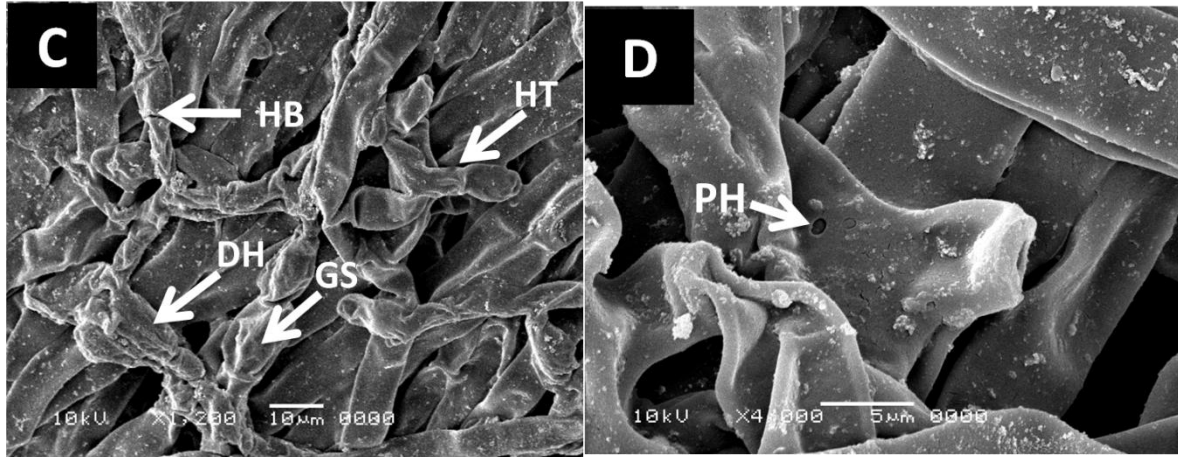
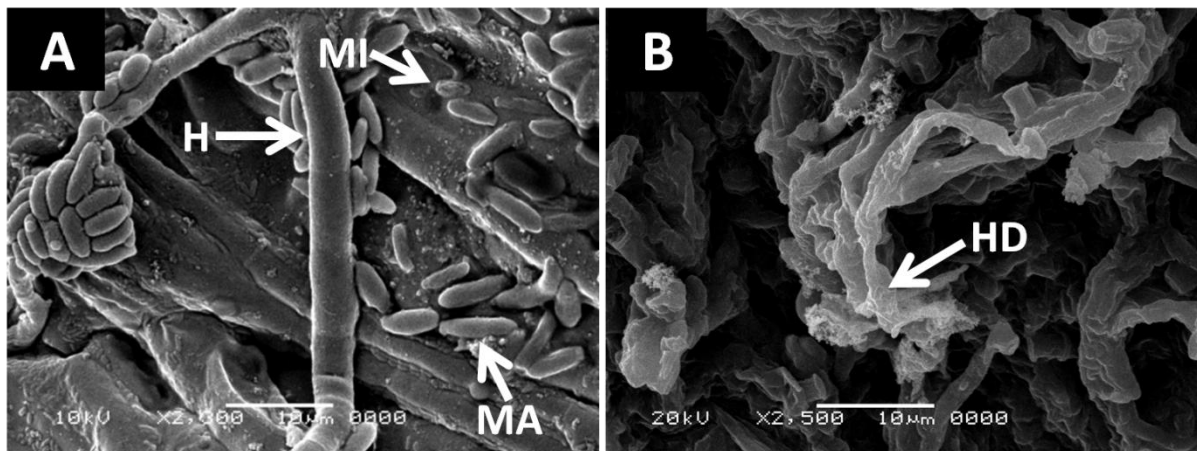


Photo 3(A, B, C and D) - SEM Micrographs of *R. solani* at x1000, x1200 and x4000. Long, even and round hypha shown in untreated ones photo 3 (A).Photos 3 (B, C and D) Showing treated hyphae culture; *R. solani* following treatment show hyphal fusion (HF), distorted hyphae (DH) and breakdown (HB) Photo 3(B and C), treated culture are generally characterized by curling and twisting (HT), Photo 3 (D) penetration hole (PH).globular structures (GS) of various sizes along the surface of the mycelia Photo 3(C).

After treatment with the tested extract, Photo 3 (B, C and D), the prominent morphological changes appeared; sparse and asymmetric mycelium, hyphal swelling, curling (HT), rough cells with wrinkle cell along the surface of the mycelia and cell distortion (DH). The integrity of the cell surface of *R. solani* began to disintegrate, pronounced collapse and loss of turgor of *R. solani* hyphae were among the typical features of advanced alteration observed with signs of cell wall breakdown and destruction (HB). Additionally, fusion among the hyphae (HF) was observed. Close up SEM examination of *R. solani* at (x4000) show evidence of penetration holes (PH) and extensive cell damage.



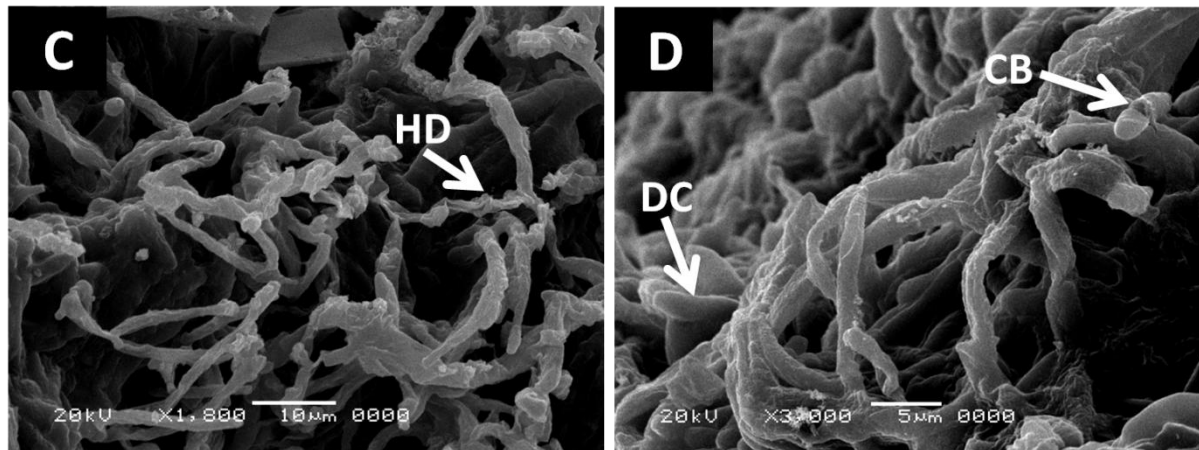


Photo4(A, B, C and D) - SEM Micrographs of *F. oxysporum* at x1800, x2300, x2500 and x3000. In untreated culture, photo 4(A) Showing uniseriate and uniform hypha (H) with macro (MA) and micro conidia (MI). Photo 4 (B, C and D) - SEM Micrographs of *F. oxysporum* co-cultured with treatment Showing hyphal disruption and break down (HD) Photo 4(B and C) with macro and micro conidia were deformed (DC) and broken down (CB) Photo 4(D) partially with reduced number Photo 4(B, C and D respectively) in comparison with control Photo 4 (A).

SEM examinations of *F. oxysporum* also showed varied morphological changes between treated and untreated mycelia; control *F. oxysporum* hyphae (H) are smooth surface, uniseriate and uniform bearing macro (MA) and microconidia (MI) as illustrated in Photo. 4(A).

AR compost tea+OP+MO treatment altered the morphology and conidia of *F. oxysporum* f. sp. *lentis*; in Photo 4(B, C and D) the mycelium appeared distorted (HD), showing extreme shrinkage, shriveling, with discontinuous areas due to the effect of hyphae breaking Photo. 4 (B and C) and cytoplasmic loss when compared with hyphae grown in control. Furthermore, macroconidial shape was greatly distorted (CD), and microconidia were broken (CB) and completely absent. As well as reduction in the amount of conidia was observed.

#### 4. Discussion:-

This study focus on biological control approach using compost tea in reducing some diseases pathological occurrence. Efficacy of compost teas and their nutrient content differs from each others, and this may be due to the differences in procedures used for preparation of compost teas, the source, nutrient composition, quality and maturity of the compost (Al-Dahmani et al., 2003).

The pH values of the four tested compost tea (6.92-8.05) were within the range suitable for compost tea preparation (Bord na Mona, 2003). Adegun loye et al. (2007) reported that most of the microorganisms in compost best survived under neutral pH. The increase in pH is likely due to an overall uptake of more anions than cations by the microbiota (Yan et al., 1996). With respect to EC, our results for AR and AR+OP compost tea recorded 2.50 ds/m and 3.30 ds/m respectively, both were within the range from 0-3.5 ds/m which is acceptable for general crop

growth (Rynk et al., 1992), while MSW and MSW+OP compost teas have higher EC values recording 6.62 ds/m and 6.33 ds/m respectively, which may be due to excess salt concentration which might hinder plant growth by affecting the soil-water balance. Lasaridi et al. (2006) proposed that value 4.0 ds/m for EC is a level considered tolerable by plants whereas values from 6 to 12 ds/m indicating toxicity due to salts for most plants up to Greek standards.

Concerning macro and micronutrients, our results shows that all compost teas contain a considerable amount of nutrients. Chemical properties are very important factor in determining the value of the produced compost tea (Hegazy et al., 2015). Enriched compost teas showed high characters than the unenriched types.

The ratio of optical density at 465 nm/ 665 nm ( $E_4/E_6$  ratio), all values in this study are relatively lower than 5 which indicate a relatively high degree of condensation of aromatic humic constitutes (HA's) (Pare et al., 1997), and showed that all compost teas are characterized with humic acids more than fulvic acids which in turn indicate enhanced humification. These results are similar to those obtained by (Claus et al., 1999).

The Microbial levels in all tested compost teas were in the range of  $10^6$  and  $10^8$  cfu/ml which is consistent with the microbial analysis of liquid biofertilizers produced from different plant-based raw materials during fermentation (Ngampimol & Kunathigan, 2008).

Scheuerell and Mahaffee, (2004); Diánez et al., (2007) have studied the level of microbial population in compost teas necessary to determine their phytopathogens suppressive level. As well as Sylvia (2004) reported that compost extract containing high population of microbiota such as *Rhizobacteria*, *Trichoderma* and *Pseudomonas* spp. induce disease resistance as well as stimulate nutrient uptake, enhanced the growth and yield of crops (Scheuerell and Mahaffee, 2002; Ingham, 2005). It also contains growth hormones and anti-pathogenic chemicals such as siderophores tannins and phenols (Antonio et al., 2008), and vitamin C (Ha et al., 2008).

Regarding the recorded Dehydrogenase activity (133.6, 125, 131.8 and 130  $\mu\text{g TPF/ml/24hrs}$ ) their high values might have been the result of high microbial activity (Benito et al., 2003). The plant biological assay revealed that the four prepared compost teas in the present study were not phytotoxic and suitable for utilization with more than 80 % of germination index (Emino & Warman, 2004).

Concerning the inhibitory effect of compost teas on the tested fungal pathogens, in vitro assay results revealed that all tested Aerated Compost Teas (ACT) exhibited significant inhibition of mycelia growth of *R.solani* and *F. oxysporum* in comparison with control. By enrichment of compost with both Olive Pomace (OP) and Microorganisms (MO), and monitoring their effect against *R.solani* and *F. oxysporum* in vitro; the recorded results showed that compost tea plus OP and MO significantly increased the antifungal activity against *R.solani* and *F. oxysporum* compared to compost tea alone. This may be due to the biological interactions between microorganisms, compost and plant products and can be a valuable source of new and

effective antimicrobial substances, which could affect differently on the microbial pathogens compared to other conventional antimicrobials (**Mokhtar et al., 2014**). This could be explained by the presence of antagonistic microorganisms either normal inhabitant of compost tea or added; Bio-agents produce biologically active compounds (antibiotics and toxic substances) that have antifungal activity, besides bioactive compounds including plant growth regulators like gibberellin, auxin, cytokinin, ethylene, abscisic acid, jasmonic acid, protein, vitamins and minerals (**Noble and Coventry, 2005**), and active antifungal compound in compost extracts (**El-Masry et al., 2002**), like OP which cause significant reduction in mycelia growth of the pathogen on PDA. The antifungal effect of OP extract is related to their chemical composition (is attributed mainly to the chemical constituents phenol and oleosidic compounds which has potent antimicrobial properties) (**Markin et al., 2003; Pereira et al., 2006; Medina et al., 2009 and Dayan et al., 2009**). Phenolic compounds have several mechanisms of antimicrobial effect such as denaturation of enzymes, this is attributed to the presence of phenolic OH group that are known to be reactive and form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in deactivation of enzymes in fungi (**Furneri et al., 2002; Velluti et al., 2003; Alma et al., 2007**), lead to inhibition of spore germination, alter the permeability of the cell wall (**William, 2008**), bind to substrates such as minerals, vitamins and carbohydrates making them unavailable for microorganisms (**Stern et al., 1996; Shahidi & Naczk, 2004**).

Obtained results showed that the fungicide Rhizolex-T inhibited the mycelia growth of *R. solani* (83.70 %, 100% 100% and 100%) and *F.oxysporum* (5.80%, 12.01%, 17.81% and 37.47%) at concentrations 1, 5, 10 and 25 ppm respectively. This fungicide is considered very effective against *R. solani* than *F. oxysporum*. The antifungal Rhizolex-T greatly effect on the lipid and membrane synthesis of pathogenic fungi. These results are in agreement with those reported by (**Abd El-Aziz, 2007; Karlsson et al., 2014**). These results are supported by that of **Hameed (2008)** who showed that Rhizolex-T completely caused growth inhibition of *R. solani*. Similar results concerning the response of Rhizolex-T at different concentration were also reported by (**Abdel-Kadar, 1997, 1999**). As revealed by SEM micrographs, morphological distortions in *R. solani* hyphae may be attributed to the occurrence of cell wall injury and/or reduction in fungal cell turgor pressure which have been suggested to be due to alterations in membrane permeability (**Rittiwong et al., 2011**), resulting in collapse and shrinkage of hyphae and cells (**Bi et al., 2006; Li et al., 2009; Liu et al., 2010**). Alterations and damage on vegetative hyphae have been previously described by many workers (**Hashem, 2011; Khan and Ahmad, 2011**); they found that the morphological changes might result from the destruction of organelles in the endomembranes system.

Regarding *F. oxysporum* SEM micrographs, the morphological distortions in hyphae and macroconidia was observed which indicate that this filtrate caused the damage of cell wall, plasma membrane of hyphae, macroconidia and microconidia. This damage may responsible for the growth inhibition of *F. oxysporum*.

Generally this indicates that AR+OP+MO extract possessed marked antifungal property against both *R. solani* and *F. oxysporum*; primarily affected cell permeability through direct interaction with the cell membrane. A change in cell permeability which might have resulted in an imbalance in intracellular osmotic pressure, subsequent disruption of intracellular organelles, leakage of cytoplasmic contents and finally cell death (Abdel-Monaim et al., 2011; Kagale et al., 2011).

## 5. Conclusion:-

These results demonstrated that compost teas enriched with OP and bioagents mixture are suitable products for in vitro suppression of some phytopathogenic fungi and thus could reduce the need of the fungicide use. The disposal of OP represents a challenge for the environment, although it might also be an opportunity; this study addressed the use of low amounts of OP wastes, which can be used to counteract the effects of some soil-borne pathogenic fungi in agriculture. Successful development of such compounds as antifungal would not only provide a potent tool for control of lentil root-rot and wilt, but also could promise success in multipurpose biorational alternatives to conventional fungicides for the management of other plant diseases. This effective, environmental friendly and human safety products has much potential.

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### الملخص باللغة العربية

التأثير المختبري لشاي الكمبوست المحضر من المخلفات الزراعية ونفايات البلديات الصلبة على بعض الفطريات الممرضة

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تم تجهيز مستخلص هوائى لشاي الكمبوست من اربع انواع مختلفة من الكمبوست الصلب ؛ من المخلفات الزراعية، (AR) من المخلفات الزراعية مخصب بتقل الزيتون (AR+OP)، من النفايات البلدية الصلبة، (MSW) من النفايات البلدية الصلبة المخصب بتقل الزيتون (MSW+OP)، و تم توصيفهم تحليليا ثم اختبار النشاط العدائى لكل منهم فى المختبر أما بمفردهم أو مع اضافة خليط من الكائنات ذات النشاط البيولوجى ضد كل من فطر الفيوزاريم اوكسيسبورم (*F. oxysporum*) و الريزوكتونيا سولانى (*R. solani*) بالمقارنة مع مبيدات الفطريات الكيمايائية. وقد تم التقييم من حيث نسبة تثبيط نمو الخيوط الفطرية ثم فحص العينة الاكثر تأثيرا بالمجهر الالكترونى لشرح طريقة عملها فى المكافحة البيولوجية ضد المسبب المرضى. و قد أظهرت النتائج مستويات عالية للعناصر الغذائية و المحتوى الميكروبي فى كل معاملات شاي الكمبوست. اعطت المعاملات المختلطة أعلى نسبة تثبيط مقارنة بالمعاملات الاخرى ضد كل من الفطريين الممرضين ، حيث اظهرت نسبة 63,52% و 44,07% فى حالة استخدام كمبوست المخلفات الزراعية المخصب بتقل الزيتون و كائنات الدقيقة ذات النشاط الحيوى (AR+OP+MO) و 57,41% و 43,89% فى حالة كمبوست المخلفات الصلبة المخصب بتقل الزيتون و الكائنات الدقيقة ذات النشاط الحيوى (MSW+OP+MO) الريزوكتونيا سولانى و الفيوزاريم اوكسيسبورم على التوالى. اظهرت نتائج الفحص الالكترونى للخيوط الفطرية للعامل الممرض التى تم ازالتها من منطقة المواجهة حدوث تغير مورفولوجيا شادا لكلا الفطريين الممرضين مثل الانكماش ، تجعد، فقدان التناسق فى الخيوط الفطرية ، حدوث تشوهات جزئية و تحلل، تقدم هذا لنتائج دليلا على أن هذا المستخلص لها لقدرة على أن يصبح مرشحاً جيداً للسيطرة البيولوجية كبديل محتملة لتطبيق المبيدات الفطرية الاصطناعية، وكمحفز نباتى فى إنتاج المحاصيل، لتحقيق الاستدامة البيئية وسلامة الأغذية.