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BIODEGRADATION OF OLIVE CAKE AND UTILIZE THE FINAL PRODUCT TO REDUCE FUNGAL INFESTATION OF SOYBEAN SEEDS WITH *Colletotrichum dematium*

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ABSTRACT

In this study, olive cake (OC) was processed under solid state culture conditions with five bioagent strains (*Trichoderma viride*; *Trichoderma reesei; Bacillus badius; Bacillus subtilis* and *Streptomyces antibioticus*) with highly enzymes activity and the effect of this microbial culturing on biochemical changes was evaluated. A significant decrease in C/N ratio from 72.45 to 25.3 was obtained. Soybean plants exhibited an enhanced defensive capacity against the *Colletotrichum dematium* pathogen in case of using composted olive cake amendment to soil as compared with control plants grown on native soil. Interestingly, the pathogen was not able to penetrate and colonize the root tissue to cause damping-off for sprouts. Moreover, combination between chemical treatment and compost amendment was also more efficiency to protect the soybean plants than chemical treatment. Our data strongly indicate that induction of soybean plant defense response is the main mechanism of biological control mediated by the OC compost.

Key words: Olive cake - Solid-state fermentation - Enzyme activity – Cellulase – Protease – Dehydrogenase – Soybean - *Colletotrichum dematium* - Plant disease.

INTRODUCTION

Spain, Greece, Italy, Tunisia and Turkey are important olive oil producers in Mediterranean basin, which represents 97% of olive oil

production in the world (López-Villalta, 1998). Olive cake is a sub-product of the mechanical olive oil extraction industry, which consists of pit and pulp of the olive fruit, olive oil and vegetable water (Nalan and Akgun, 2005). Mona (2003) stated the biochemical constituents of olive cake recorded about 8.20, 21.0, 22.8, 5.35, 33.0 and 0.8% of moisture, cellulose, lignin, crude proteins, total organic carbon and total organic nitrogen (%), respectively and 41.20 of C/N ratio (Cammarota and Freire, 2006).

The principal requirement of a compost materials to be safely efficiently used as an organic fertilizer is stability or maturity, which implies conversion of organic matter to a more refractory, i.e. stable form, minimum content of phytotoxic compounds and absence of plant and animal pathogen (Jimenez and Garcia, 1989).

C/N ratio is considered to be the most important aspects of composting, since the microorganisms involved require carbon for energy, and nitrogen for protein synthesis. The rate of decomposition is affected according to C/N (Gaur, 1987). A balanced C/N ratio usually ensures that the other required nutrients are present in adequate amounts. Also, addition of raw materials blended to provide a C : N ratio of 25 : 1 to 30 : 1 are ideal for active composting, although initial C/N ratios from 20 : 1 up to 40 : 1 consistently give good composting results. It has been stated that when the C/N ratio is less than 20, the compost is mature and can be used without any restrictions (Sunlla and Gaur, 2003).

Nektarios *et al.* (2005) examined the root-infecting fungal pathogen *Fusarium oxysporum* f.sp. *radicislycopersici,* which was used to inoculate the roots of tomato plants (*Lycopersicon esculentum* Mill.) grown on a compost mix made from grape marc wastes and extracted olive press cake (GM-EPC). Plants exhibited an enhanced defensive capacity against the pathogen as compared with control plants grown on peat. Interestingly, the pathogen was not able to penetrate and colonize the root tissue. Moreover, the sterilized compost extract was also able to protect the plants. These data strongly indicate that induction of plant defense response is the main mechanism of biological control mediated by the GM-EPC compost. This destruction of plant pathogens is not only caused by heat but it's a combination of factors including: 1) Competition for food from compost microorganisms; 2) Inhibition and antagonism by compost

microorganisms; 3) Consumption by compost organisms; 4) Biological heat generated by microorganisms.

The present work was carried out to study the utilization of solid state fermentation of olive cake and it's role in control anthracnose disease in infested soybean seeds which caused by *Colletotrichum dematium,* using composted olive cake with three levels of addition with and/or without Topsin-M 70 as chemical treatment in comparison with control (inocula-free seeds).

MATERIALS AND METHODS

Preparation of compost heap from olive cake residues:

1- Preparation of raw materials:

Five hundred kilogram of olive cake as organic plant residues was obtained from Local Labor Productivity Unit, El-Safe Center, Giza, Egypt. All this amount used for *in-vitro* (Solid-state fermentation of olive cake residues) and *in-situ* (greenhouse) experiments.

All olive cake was chopped to a small portions and air dried, then re-dried in an oven at 60°C over night. The dried cake was grinded in particle seize about 0.5 – 2.0 mm (include dried pulp and crashed stone of olive fruits), sieved through standard-mesh sieves to obtain particles that passed through a 40 mesh screen.

1.2. Solid-state fermentation of olive cake residues:

1.2.1. Enrichment of bioagent strains:

A strain of *Trichoderma viride* EMCC 107c and *Trichoderma reesei* were grown and maintained on potato dextrose agar (Riker and Riker, 1936), *Bacillus subtilis* and, *Bacillus badius* strain were grown maintained on nutrient glucose agar (Dowson, 1957) and *Streptomyces antibioticus* strain was grown on Jensen's agar medium (Allen, 1950).

1.2.2. Estimation of enzyme activities for selected strains of fungi, bacteria and actinomycetes:

1.2.2.1. Estimation of cellulase activity:

The available method to estimate cellulase activity of selected strains of fungi, bacteria and actinomycetes, is based on the determination of either released reducing sugars or evolved CO_2 after the incubation of fungi and bacteria or actinomycetes with Carboxy methyl cellulose (CMC) for 24 hours at 50°C. CMC was used as a substrate for cellulase enzyme according to the method described by Schinner and Von-Mersi (1990).

Fungal strains was cultured on basal nutrient medium (Kvachadze and Yashvili, 1996). Carboxy methyl cellulose activity was determined according to the method of Somogyi (1952). Moreover, bacterial strains was spread on Difco nutrient agar plates (Difco, 1985), then Carboxy methyl cellulose activity was described according to Moatza *et al.* (1998). On the other hand, streptomyces strain enriched on basal medium according to Waksman (1961), then cellulase activity was assayed as mentioned in case of fungi and bacteria. The enzyme activity was determined using pure glucose at various concentrations served in preparing the standard curve.

1.2.2.2. Estimation of protease activity and soluble protein:

Proteinase activity of microbial isolates was determined according to Ladd and Butler (1972) based on determination of amino acids released after incubation of the microbial isolates with sodium caseinate for 2h at 50°C using Folin Ciocalteu reagent. Fungal isolates were grown on basal nutrient medium but 0.49g/L casein was added instead of carbon source, while bacterial isolates was grown on liquid medium as described by Moatza *et al.* (1998) and actinomycetes isolate was grown on starch-casein medium as described by Kuster and Williams (1964). The enzyme activity was determined using pure tyrosine at various concentrations, which served in preparing the standard curve.

Also, soluble protein was determined according to Lowry *et al.* (1951), using bovine serum albumin at various concentrations, which served in preparing the standard curve.

1.2.2..3. Dehydrogenase activity:

Dehydrogenase activity of microbial filtrates was determined according to Skujins (1976), using tri-phenylformasan (TPF) at various concentrations, which served in preparing the standard curve.

1.2.3. Adjustment moisture content of olive cake:

Moisture content of tested olive cake was determined according to the method of A.O.A.C. (1990) and adjusted to the required moisture level (60-70%) by sterilized distilled water.

1.2.4. Cultivation procedure:

Bioagent inoculum was prepared by inoculating conical bottles (500 ml capacity) containing 200ml of slant enrichment media. The culture spores were harvested after 10 days (for fungal or actinomycetes strains) and/or 48 hr (for bacterial strains) of incubation at suitable temperature for each strain. Spore suspension of about

5.2×10^6 C.F.U./ml for fungal strain, 2.3×10^6 C.F.U./ml for bacterial strain and 2.3×10^6 C.F.U./ml for actinomycetes were used to inoculate the sterilized tap water containing selected cellulosic raw materials as the only carbon source.

In all laboratory experiments Erlenmyer flasks (500ml) containing 20 g of moisted olive cake. All flasks were autoclaved at 121°C and 1.5 psi for 20 min. Incubation was extended for 21 days at room temperature ± 5.0°C. Samples were collected at different intervals (every 7 days) for determination of changes in biochemical parameters (El-Tahan, 1995).

1.2.5. Evaluation of the changes in biochemical parameters and microbial total count of solid state fermented material:

1.2.5.1. Total microbial count:

Total microbial count of different microbial group i.e. bacteria, actinomycetes and fungi were determined. Using the dilution plate technique using specific enrichment media. The plates were incubated for 7 days at 30°C in case of fungi and actinomycetes, but for 48 hr in case of bacteria at 30°C, then the number of colony was recorded.

1.2.5.2. Biochemical changes of olive cake during solid state fermentation:

All samples which used for chemical analysis was prepared as follow: the fresh samples was air dried, then dried in an oven at 60°C/over night, then became ready for the rotation examination of major constituents after 0, 7, 14 and 21 days.

- pH and electrical conductivity (EC) were determined in water extract suspensions (1:5, w/v) using a pH meter according to Sonneveld and Beusekom (1974).
- Organic carbon was determined according to the method which described by Cottenie *et al.* (1982).
- The total organic nitrogen (total nitrogen) content in dried samples was determined by using micro-Kjeldahl method according to Cottenie *et. al.* (1982) and A.O.A.C (1995).

1.2. Greenhouse experiment (*In-vivo* experiment):

A field experiment was executed in sandy soil at El-Ismailia Agricultural Research Station Agricultural Research Center to study the effect of integrated biofertilizer management on the *Colletotrichum dematium* as a soil-borne and seed-borne pathogen which cause anthracnose disease of soybean (*Glycin* max L. c.v. Crawford) plants.

- Preparation of compost heap: Heap (1 x 1 x 0.7 m) was set up by using about 200kg of olive cake supplemented with cellulose decomposers bioagents suspended in water volume efficiency to mad the water content of cake adjusted to 60-70%. The heap was turned up each 10 days up to 80 days.
- Preparation of inoculated soybean seeds (c.v. Crawford) treatment using technique of seed infestation described by Abdel-Moity (1985) using spore suspension (5×10^6 spore/ml).
- Topsin-M 70 (Table, 6) fungicide was applied by dressing soybean seeds (c.v. Crawford) which were contaminated with the *C. dematium*, after infestation process seeds was left to dry, then dressed with the tested fungicide at recommended dose (3.0g/kg seeds).

1.3. Pot experiment of soybean.

A pot experiment was carried out during summer season of 2005. Dry treated seeds of soybean (*Glyin max* L. c.v. Crawford) was sown in pots (poly-ethylene pot 30cm diameter) at the rate 10 seed/pot, each pot containing 10kg of sterilized sand soil (by fumigated with formaldehyde 30% and solarization for about 30 days), that taken from surface layer 0-20 cm depth of Ismailia Experimental Research Station. Before seeds sowing, composted olive cake was added and mixed with the soil according the following treatments: Control (A): Inocula-free seeds were sown as a control; Control (B): Infested seeds was set in native soil without any addition; Control (C): Infested and chemical treated seeds by examined fungicide (Topsin-M 70) was set in native soil without any addition; B + 1% Compost per pot; B + 2% Compost per pot; B + 3% Compost per pot; C + 1% Compost per pot; C + 2% Compost per pot; C + 3% Compost per pot.

Pots were arranged in the greenhouse in a complete randomized block design with three replications for each treatment. During the experimental period, tap water was added to keep soil at moisture content at ca 70% of water holding capacity. Percentage of pre- and post-emergence damping off as well as healthy survivals percentages in each treatment was determined 2 and 6 weeks after sowing using the formula according to El-Helaly *et al.* (1970).

2. Statistical analysis:

The statistical analysis was computed using analysis of variance procedure described by Sendecor and Cochran (1980), the significant

mean differences between treatment means were separated by Duncan's Multiple Range Test (Duncan, 1955).

RESULTS AND DISCUSSION

1. Estimation of enzyme activities for selected strains of fungi, bacteria and actinomycetes to biodegradation of olive cake through solid state fermentation:

Results in Table (1) showed the diversity of cellullytic and lipolytic enzymes activity among the microbial species of used bioagent. The screening of bioagent species for cellulase potentiality revealed that *Trichoderma ressei* represented the highest CMC ase and filter paper ase activity, which recorded 3.87 and 4.00 µmole/ml/min for CMC-ase and filter paper cellulose, respectively.

In the same manner data in Table (1) showed that, the best isolate of tested bioagent strain which were tested their extraction to soluble protein and activity for protease enzyme was *Bacillus subtilis*, which gave the highest crude soluble protein content (461.83µg/ml) and also recorded the highest protease activity (0.0221 U/ml/min).

On the other hand, results in Table (1) showed that, *streptomyces antibioticus* exhibited the highest activity for dehydrogenase of five bioagent isolates which was recorded 305.33 TPF µg/ml.Results in Table (1) indicated that the ability of various species of bacteria, fungi and actinomycetes to produce extracellular enzymes when they grow on cellulosic substrates. Our findings are in agreement with Kang *et al.* (2004) they reported that, many microorganisms either bacteria or fungi have been recorded as capable of producing cellulase enzyme which allow them to utilize cellulose materials as source of energy. Many actinomycetes have been reported to have less cellulase activity than bacteria and fungi, species of *Trichoderma reesei* and *Tricoderma viride* are considered to be the best cellulase producers. While mesophilic actinomycetes, are regarded to produce cellulases and hemicellulases i.e. *Streptomyces antibitocus*, and *Streptomyces roseus* (Charest *et al.*, 2004). Also, many bacterial strains have been produced of cellulases through composting process i.e. *Pseudomonas fluorescens*, *Bacillus subtitis*, and *Bacillus brevis* (Baeta-Hall *et al.*, 2005).

Table (1): Enzymes activity of different selective fungal, bacterial and actinomycetes species isolates, which using in decomposing process of olive cake by solid state fermentation.

Isolates	CMCase	Fpase	Soluble protein	Protease activity	DeHase activity
Trichoderma viride	3.47 [b]	3.61 [b]	266.48 [c]	0.0132 [c]	161.60 [b]
Trichoderma reesei	3.87 [a]	4.00 a	278.14 [c]	0.0137 [c]	171.40 [b]
Bacillus badius	1.27 [d]	0.66 [c]	371.44 [b]	0.0177 [b]	63.68 [c]
Bacillus subtilis	1.52 [c]	0.76 [c]	461.83 [a]	0.0221 [a]	74.14 [c]
Streptomyces antibioticus	1.30 [d]	0.67 [c]	193.59 [d]	0.0098 [d]	305.33 [a]
L.S.D. (0.05)	0.210	0.200	26.83	0.001	14.565

- Each value represents the mean ± S.D (Standard Division) and mean of three replicates.
- Values in the same column with the same letter are not significantly at ($p \leq 0.05$).
- CMCase: Carboxy methyl cellulase µmol/ml/min; Fpase: Filter paper ase µmol/ml/min; Soluble protein: µg/ml; Protease activity: Unit/ml/min; DeHase activity: Dehydrogenase activity, TPF µg/ml culture filtrate.

It was obvious that bacteria represented particularly in *Bacillus subtilis* is considered to be the optimum for producing soluble protein and protease activity more than both of fungi and actinomycetes.

These findings are the same those who obtained by Charest *et al.* (2004) they also added that the microorganisms were the mesophlic fungi i.e., *Trichoderma reseei*, mesophilic bacteria (i.e. *Bacillus subtilis*) and mesophilic actinomycetes (*Streptomyces antibioticus*) was reported to produce proteolytic enzymes. On the contrary, although many investigators reported that, the production of neutral protease using agro-industrial residues as substrate in solid-state fermentation as olive oil cake, such processes would help in reducing the cost of production and pave the way in effective solid waste management (Chandran *et al.*, 2006).

The increase in dehydrogenase activity was attributed to intense activity of actinomycetes isolate than each other species of bioagent. The present results are in harmony with those of Vuorinen and Saharinen (1999) and Osama (2005), they reported that dehydrogenase activity influenced by the bacterial, fungal and actinomycetes populations. The initial high dehydrogenase activity might have been the result of high microbial activity due to the high composting contents (high organic matter) and consequently the decrease coincided with the decrease in compost content; so the

dehydrogenase activity decreases with composting time of different organic feedstock and remained stable after 2 or 3 months of the process. Whereas, Caravaca *et al.* (2006) stated that the addition of both organic amendments, particularly fermented dry olive cake, decreased significantly the dehydrogenase and protease activities.

2. Effect of different incubation periods on total microbial count during solid state fermentation process of olive cake:

Data presented in Table (2) showed that the number of bacteria was increased at the beginning of the aerobic composting cycle, while it was markedly decreased in the third day of fermentation. However, it was increased at the end of fermentation process (cooling phase). The same trend of the results was also observed with fungi and actinomycetes.

Table (2): Microbial count during incubation periods up 21 days of solid state fermentation for inoculated olive cake at room temperature.

Incubation period (day)	Total microbial count Log numbers (-×10^5 CFU/g fresh weight basis)		
	Fungi	Bacterial	Actinomycetes
0	6.09 ^{c}	9.17 ^{a}	6.50 ^{b}
3	9.54 ^{a}	8.22 ^{a}	9.75 ^{a}
6	6.20 ^{c}	8.11 ^{a}	6.50 ^{b}
15	6.78 ^{b}	8.05 ^{a}	2.95 ^{c}
21	6.50 ^{bc}	8.74 ^{a}	9.40 ^{a}
L.S.D. $_{(0.05)}$	0.541	n.s. 1.279	0.564

- Data are expressed as No. of colony/agar plate and one mean of 4 replicates; CFU: Colony forming unit.
- Each value represents the mean ± S.D (Standard Division).
- Values in the same column with the same letter are not significantly at ($p \leq 0.05$).

Our results are in agreement with those added that increasing biological activity at the beginning of composting process caused an increase in the temperature which is responsible for causing a decrease in the number of mesophilies microorganisms (Rhodes *et al.*, 1994). Mesophilic microbes were examined in plant residues compost at different stages of composting process from zero day to 28 days. Some of the mesophilic bacteria observed in initial stage of composting (i.e. *Bacillus* species). After 20 days of composting process lower species diversity of mesophilies (only *Bacillus* sp.) were isolated, which was most likely due to the high temperature (60-68°C).

Some of thermo tolerant bacteria such as *Bacillus subtilis* and *B. polymyxa* were observed at later stage of composting process (Ghazifard *et al.*, 2001). Some Actinomycetes species appear during thermophilic phase of composting i.e. *Streptomyces roseus* while other species become important during cooler curing phase (mesophilic phase) i.e. *Streptomyces* spp. (Eiland *et al.*, 2001). It has observed that mesophilic populations of bacteria, fungi and actinomycetes increased after compost temperature decline (Claire *et al.*, 2005).

3. Biochemical changes of olive cake during solid state fermentation:

The changes in pH values (1:5 in de-ionized water) was presented in Table (3). pH values (1:5) were slightly decreased in early intervals of fermentation process, then pH values increased afterwards to reach to neutral value.

Table (3): Change in biochemical parameters during solid state fermentation process (up to 21 days).

Incubation period (day)	Biochemical parameters				
	pH value	EC (μSm^{-1})	T.C.%	T.N.%	C/N ratio
0	7.60 ^{a}	6.45 ^{d}	71.73 ^{a}	0.99 ^{c}	72.45 ^{a}
7	6.41 ^{c}	9.40 ^{c}	40.61 ^{b}	1.04 ^{bc}	39.05 ^{b}
14	6.92 ^{bc}	11.62 ^{b}	33.53 ^{c}	1.14 ^{b}	29.41 ^{c}
21	7.11 ^{ab}	12.64 ^{a}	32.13 ^{c}	1.27 ^{a}	25.30 ^{d}
L.S.D. $_{(0.05)}$	0.594	0.871	4.012	0.107	3.855

- **Each value represents the mean ± S.D (Standard Division) and mean of three replicates.**
- **Values in the same column with the same letter are not significantly at ($p \leq 0.05$).**
- **EC: Electric conductivity; T.C.%: Total carbon %; T.N.%: Total nitrogen %; C/N.: Total carbon to total nitrogen ratio.**

From the results obtained the decreasing of pH values (1:5) at the early periods may due to production of simple organic acids causing further acidification. This results are in agreement with these of Kaloosh (1994) and Leal *et al.* (2006) they reported that, composting process may proceed effectively at pH levels between 5.5 and 9.0. pH values of the compost were varied due to compost origin where some of the substrates. During the first and second week of composting process a slight decrease in the pH value of compost recorded pH values of 6.5 and 6.2 this due to formation of CO_2 and organic acids by microbial activity during the process. After the second week of

composting the pH values continued to increase and rise up to 8-9 and the tendency of rising up may occurred till the end of the process. The changes in the pH values are generally attributed to the microbial flora, which attack the organic nitrogenous materials.

Table (3) showed the electrical conductivity [EC (1:5, μSm^{-1})] changes in decomposing materials, values were increased gradually till the end of the process where the average of EC (1:5, μSm^{-1}) values at the beginning of the process exhibited 6.45 μSm^{-1} and reached to 12.64μSm^{-1} at the end of fermentation process. These results are similar to those obtained by Laila (2001) who studied the evaluation of the electric conductivity (EC) during a controlled trial of composting. They found that the EC was increased progressively from the start to stability in the final phase of bio-oxidation of composting. The increase in EC may be due to the compost had a higher concentration of carboxyl and hydroxy phenolic groups. Consequently, measurements of EC are considered useful for estimating the degree of maturity (Paredes *et al.*, 2002). Manios (2004) evaluate the compost derived from a local solid olive press cake, olive tree leaves and branches have been evaluated for their behavior during composting, the quality of the end product was evaluation included both a detailed physiochemical pH, electrical conductivity (EC). The end products contained large amounts of organic matter, usually combined with an increased EC value. Pressed grape skins should be considered as the ideal raw material, producing a high quality compost, with the lowest EC value ($1.57 mS/cm^{-1}$).

Data given in Table (3) showed that the percentage of organic carbon gave a gradual decrease in solid state fermentation process period, these loss of carbon depended on the sort of substrate which formed fermented media (olive cake).

The gradual decrease in organic carbon was mostly due to the loss of carbon as CO_2 owing to microbial oxidation during the decomposing process. These findings are similar to those found by Kaloosh (1994) and Ferrer *et al.* (2001) they attributed the loss of carbon as CO_2 was also due to the presence of cellulolytic fungi and may accelerate the rate of decomposition and declined the organic carbon. They also indicated that, the decreasing of organic matter during the composting process may due to oxidation effect on the organic acids obtained under aerobic conditions. These results are also in agreement with those reported by Paredes *et al.* (2002).

Data presented in Table (3) showed that there were remarkable increases in the percentage of total nitrogen during the decomposing process from the first day to the 21th day and ranged between 0.99% and 1.27%.

Nitrogen percentage in fermented media was increased, similar findings were reported by Kaloosh (1994) and Ferrer *et al.* (2001) who indicated that the increase in total nitrogen during the decomposing process may due to reduction in the weight of composted materials. On the other hand, Gaur (1987) indicated that inoculation of plant wastes with cellulolytic fungi led to improve the nitrogen content of the compost. These results were also in harmony with those obtained by Abdel-Wahab *et al.* (2003).

From data presented in Table (3) showed that, C/N ratio sharply decreased from the period of first days to 21 days, where its average ranged from 72.45 to 25.30. From the period of fourteen days to the period of twenty one days the ratio was markedly narrow and its average recorded 29.41 to 25.30.

From the obtained results, it was concluded that the narrowing of C/N ratio with the progress of the decomposing process is a common features and an indicator for maturation of composting material.

As a result of high depletion of organic-C, the total nitrogen was increased hence, the C/N ratio was decreased. Similar findings were obtained by Ferrer *et al.* (2001) they indicated that the C/N ratio tended to be narrow with time in compost heaps. This may be due to the loss of carbon as CO_2, while the nitrogen remained more tightly bounded in organic combination as long as the C/N ratio is wide. These results are in agreement with those obtained by Gaur (1987) and Wong *et al.* (2001) they found that addition of some amendments such as inoculation with cellulololytic microorganisms led to decrease organic-C and cause narrow C/N ratio of decomposed materials.

4. Evaluation of organic soil amendment by composted olive cake to suppress soybean anthracnose caused by *Colletotrichum dematium* under green house condition:

Data in Table (4) indicate that the tested fungicide reduced percentage of disease severity by 69.60% compared with those of the positive control B (51.50%). Moreover, supplemented soil with organic amendment by composted olive cake in three levels (1, 2 and 3%) of addition cause suppression of seed-borne disease as damping-off of plants, the most effective level 3% of composted olive cake

which recorded 67.95% in comparison with chemical treatment by Topsin-M 70 (69.60%). On the other hand, combined between chemical treatment (Topsin-M 70) at recommended dose and three level of addition by biofertilizer of composted olive cake, revealed that disease severity (%) increased with increase the level of supplementation by biofertilizeres to reach 86.31% in comparison with positive control C (69.60%).

Table (4): Effect of organic amendment with composted olive cake on suppression and reduced severity of artificially infestation of soybean seeds (*Glycin max* L. c.v. Crawford) with *Colletotrichum dematium* under greenhouse conditions.

Treatment	Pre-emergence damping-off	Post-emergence damping-off	Survival seedling
Control (A)	12.30 ± 0.52 ^{ef}	2.20 ± 0.09 ^{e}	85.50 ± 3.59 ^{ab}
Control (B)	41.30 ± 1.73 ^{a}	7.20 ± 0.30 ^{c}	51.50 ± 2.16 ^{f}
Control (C)	26.10 ± 1.10 ^{b}	4.30 ± 0.10 ^{d}	69.60 ± 2.92 ^{c}
(B) + 1% compost/pot	24.01 ± 1.01 ^{c}	18.54 ± 0.78 ^{a}	57.45 ± 2.41^{e}
(B) + 2% compost/pot	22.71 ± 0.95 ^{c}	14.20 ± 0.60 ^{b}	63.09 ± 2.65 ^{d}
(B) + 3% compost/pot	18.53 ± 0.78 ^{d}	13.52 ± 0.57 ^{b}	67.95 ± 2.85 ^{cd}
(C) + 1% compost/pot	16.97 ± 0.71 ^{d}	2.80 ± 0.12 ^{e}	80.23 ± 3.37 ^{b}
(C) + 2% compost/pot	13.57 ± 0.57 ^{e}	2.43 ± 0.10 ^{e}	84.00 ± 3.53 ^{ab}
(C) + 3% compost/pot	11.75 ± 0.49 ^{f}	2.12 ± 0.09 ^{e}	86.31 ± 3.62 ^{a}
L.S.D. $_{(0.05)}$	1.625	0.690	5.239

- **Each value represents the mean ± S.D (Standard Division) and mean of three replicates.**
- **Values in the same column with the same letter are not significantly at ($P \leq 0.05$);**
- **Control (A): Inocula-free seeds were sown as a control; Control (B): Infested seeds was set in native soil without any addition; Control (C): Infested and chemical treated seeds by examined fungicide (Topsin-M 70) was set in native soil without any addition.**

The aforementioned results are closed and explain by many researches which recorded that that compost application in some cases can strongly inhibit plant pathogens and reduce infection of plants (Hoitink and Fahy, 1986). Different mechanisms have been suggested (Hoitink and Boehm, 1999), one is antibiosis, i.e. microbial formation

of antibiotic substances by compost microorganisms, suppressing the pathogen. Ylva *et al.* (2004) stated that possible biocidal effects of composts when used in cultivation may be explainable by the presence of natural toxic compounds formed during composting. Work with compost amended potting mixes or light-colored peat mixes demonstrated the suppression of foliar disease symptoms caused by *Colletotrichum orbiculare* on cucumbers (*Cucumis sativus* L.), relative to plants grown in dark peat mixes conducive to disease. Molecular and biochemical assays associated an increase in two key plant defense enzymes, b-1,3-glucanase and peroxidase, with reduced foliar disease symptoms following pathogen inoculation (Zhang *et al.*, 1996). Stone *et al.* (2003) reported that field experiments documented the suppression of symptoms of foliar brown spot anthracnose (causal agent *Colletotrichum lindemuthianum*) of cucumber grown in soil amended with composted paper mill residuals. They have further documented this phenomenon here showing foliar disease suppression in a controlled environment, they demonstrated that the foliar disease suppression observed with the composted forms of paper mill residuals was an induced form of resistance, exhibiting several characteristic features of systemic acquired resistance (Gary *et al.*, 2003).

CONCLUSION

Solid state fermentation technology using non pathogenic microorganisms which can produce hydrolytic enzymes which will be advantageous for the proper utilization of these residues. Utilization of agricultural residues through biotechnology is becoming more and more significant with the dual goal of waste disposal and value addition. Since microbial activity is the key aspect in this area, there is enormous opportunity for the cost effective production of organic matter, which is an important item in the agro-industrial. Such processes would not only help in reducing the cost of production but also pave the way in effective solid waste management. Combination of microbial decomposing olive cake and chemical pesticide proved efficient for enhancing pathogen suppression. However, in order for this microbial cultured olive cake to be of use as an organic amendment for native soil, further studies are required to ascertain its role in suppress plant pathogen.

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التكسير الحيوي لتفل الزيتون و استخدام الناتج النهائي لتقليل الإصابة الفطرية لبذور فول الصويا بكوليتوتريكم ديماتيم

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في هذا البحث تم الحصول علي تفل الزيتون الناتج من عصر الزيتون لاستخراج الزيت والذي يمثل مصدر هام من مصادر المخلفات الزراعية وذلك لاحتوائه علي مركبات لجنوسليلوزية وفينولية ومن ثم يصعب التخلص منه بالطرق الآمنة.

لذا تم استخدام بعض الميكروبات التي لها قدرة عالية علي إنتاج الإنزيمات المحللة للسليلوز والبروتين وتكون غير ممرضة للنباتات من نوع ترايكوديرما فيردي، ترايكوديرما ريزي، باسيلس سابتليس، باسيلس باديس و ستريبتومسيس انتيبيوتيكس وذلك تحت ظروف المعمل خلال عملية التخمر الصلب و تم تتبع التغيرات البيوكيميائية خلال ٢١ يوم.

فقد تم دراسة تأثير عملية التخمر الصلب علي التغير في درجات الـ pH ودرجات التوصيل الكهربي والنسبة المئوية لكلا من المحتوي الكلي من الكربون والنيتروجين العضوي وكذلك تقدير معدل الكربون إلي النيتروجين، وذلك كدلالة علي درجة نضج المخلف المتخمر.

وبناءا علي نتائج التجربة المعملية تم عمل كمر لتفل الزيتون باستخدام نفس الميكروبات الحيوية واستخدام الكومبوست الناتج بثلاث مستويات مختلفة للإضافة ١، ٢، ٣% في مقاومة فطر بكوليتوتريكم ديماتيم المسبب لمرض انثراكنوز فول الصويا والذي يكون محمولا علي البذور وذلك مقارن بالمبيد الكيميائي توبسين–م ٧٠ وايضا تم عمل معاملات متكاملة منهما معا.

و كانت النتائج في حالة استخدام كومبوست تفل الزيتون بالمعدل الأعلى للإضافة (٣%) هي ٦٧,٩٥ % بينما كانت ٨٦,٣١ % في حالة استخدام هذا المعدل من الإضافة مع الجرعة الموصي بها من المبيد الكيميائي توبسين–م ٧٠.