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Research Article

Control of Fusarium Wilt of Tomato Caused by *Fusarium oxysporum* F. Sp. Radicis-Lycopersici Using Mixture of Vegetable and *Posidonia oceanica* Compost

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A compost of vegetable waste and *Posidonia oceanica* mixture (70 : 30% vol : vol) was tested *in vitro* and *in vivo* for its efficacy against *Fusarium oxysporum* f.sp *radicis-lycopersici* (Forl), the causal agent of Fusarium wilt of Tomato (*Lycopersicon esculentum* cv. chourouk).The incorporation of non-sterilized VPC in the culture medium showed potent antifungal activity against Forl and complete inhibition of mycelium growth was observed for all the tested compost rates (0.5, 1, 2, 4, 6, 8, 10, 15 and 20%). However, only the highest rates (15 and 20%) of a sterilized suspension of VPC were effective in preventing mycelial growth. Nine indigenous bacterial strains isolated from VPC exhibited antagonism against Forl. Based on 16S rDNA sequence analysis, the isolates were assigned to *Bacillus sphaericus* (B12 and BS2), *Pseudomonas putida* PPS7 and *Burkholderia gladioli* BuC16. Under green house condition, seed inoculation by B12, BS2, PP7 and BuC16 strains protected significantly tomato against *Fusarium oxysporum* f.sp *radicis-lycopersici* (Forl) attacks.

1. Introduction

During the last decades, various studies in Tunisia investigated the effects of organic composts as nutrient-rich amendments to correct mineral deficiencies of soils in a semiarid climate [1], In this context, we have conducted some studies, aimed to exploit the ability of Municipal Solid Waste (MSW) as an organic feedstock to be transformed into compost as well as the mineralization of MSW when added to soil [1–3]. The evolution of microbial biomass was studied by [4, 5]. Application of composted Municipal Solid Waste Compost (MSWC) can lead to addition of potentially toxic heavy metals to Tunisian soils [6]. Some compost may also contaminate the soil with aflatoxins [7]. Other sources of organic matter could be used as alternatives to MSWC. In Tunisia vegetable garden waste was not considered for compost production. Also, the use of compost as a biocontrol agent able to limit some plant disease has not been reported from Tunisia.

It is well known that compost offers a disease control alternative to fungicides [8]. The use of composts to suppress soil-borne plant pathogens has been extensively reviewed by several authors [9]. Different mechanisms have been postulated to control plant diseases by compost application such as competition for nutrients, antibiotic production by beneficial micro-organisms, activation of disease-resistance genes in plants [10], triggering systemically acquired resistance mechanisms [11] and compost obtained from heterogeneous vegetable wastes [12]. Pascual et al. [13] showed important suppressive effects against disease caused by several plant pathogens such as *Phytophthora* spp. [10, 14], *Rhizoctonia spp*. [15], and *Fusarium spp*. [16]. Also, *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Streptomyces* spp., and other

bacterial genera, as well as *Penicillium* spp., *Aspergillus* spp., *Trichoderma* spp., *Gliocladium virens*, and other fungi have been identified as biocontrol agents contained in compost. Hardy and Sivasithamparam [17] showed important suppressive effects against diseases caused by several plant pathogens such as *Pythium* spp. Moreover, compost is biodegradable, safe to apply, and less expensive to develop than fungicides.

Soil sterilization destroys the most heat-labile part of compost microbial communities and temporarily reduces microbial activity [18]. Manipulation of agricultural systems, through additions of composts, green manures, and cover crops is aimed at improving endogenous levels of general suppression [19]. This generalized improvement in microbial activity due to the addition of green composts may be another limiting factor to the establishment of plant pathogens into soils [20]. In addition, individual species of bacteria or fungi antagonisms could be related to extracellular metabolites released in the culture medium [21, 22], which act as the functional elements of the autochthonous microflora in suppressive composts. A key role in the suppressive effect of composts against root pathogens has also been attributed to the microbial activity of composts [23].

Tomato plants treated by *Pseudomonas putida* PPS7 were characterized by a percentage of healthy plants ranging between 78 and 83.6%. *P. putida* is well known BCA for their suppressive effects; this may be due to various mechanisms such as the chelation of iron by siderophore [24, 25]. De Boer and Kindt (2006) [26] found that the role of siderophores was associated with the antagonistic properties of *Pseudomonas putida* WCS358 in suppressing Fusarium wilt of radish.

*Bacillu*s spp. are attractive candidates for use as BCA, because of their capacity to produce a wide variety of active metabolites, their abundance in soil, and their ability to form endospores [27]. Parasitism is expressed through degradation of the cell walls of pathogenic fungi and relies on production of extracellular lytic enzymes. For example, several *Bacillus* species such as *B. circulans* [28]; *B. licheniformis* and *B. cereus* [29]; *B. pabuli* [30] and *B. pumilis* [31] produce enzymes that degrade chitin, the insoluble linear polymer of β-1,4-N-acetylglucosamine (GlcNAc), which is the second most abundant polysaccharide in nature and a major component of most fungal cell walls.

Fusarium crown and root rot is an important soil-borne disease, with the potential to limit productivity in glasshouse and field tomato crops. The causal agent, *Fusarium oxysporum* f. sp. *radicis-lycopersici* (*Forl*) race 0, was found in Japan, but was subsequently identified in many other regions, including USA, Canada, Europe, and Israel [32].

Increased early injury to the roots and collar of tomato plants caused by *Forl* was also observed in Tunisia [33], where yield losses were reported to range between 20 and 60%.

Fungicides are of little use on most Fusarium diseases [34]. *Forl* widely distributed in soil is known as a plant pathogen. It is also known to be the first eukaryotic denitrifier that catalyzes the reduction of nitrate into gaseous nitrous oxide (N_2O) [35]. In fact, *Forl* contributed to denitrifying soil.

Biological control of Fusarium wilts, in the form of natural microbial populations in soils, has been recognized for over 70 years [36]. The potential of compost for the control of crown and root rot CCR of green house-grown tomato caused by *Forl* was investigated by [37] who showed the suppressive effect on Fusarium wilt using various composts not involving Posidonia as organic matrix in tomato cv. *Marmande*.

The overuse of chemical fertilisers and excessive disturbance often leads to soils low in soil organic matter (SOM). The levels of SOM in Tunisian soils have been declining sharply in the last decades, which increased the soil degradation. Municipal solid compost could bring some pollutants like heavy metals into soil [1]. As alternative to municipal solid compost, we propose the use of wastes or *Posidonia* residues.

Posidonia oceanica is the main sea grass in the Mediterranean countries, as such Tunisia and provides substrates to a species-rich epiphytic community, which achieves maximum biomass between the end of spring and the end of summer [38], covering over 50000 km^2 [39]. As [40] indicated, the disposal of the annual accumulation of *P. oceanica* on the beaches of the Mediterranean causes a series of economic and environmental problems. In this particular situation, the leafy deposits of *P. oceanica* on the beach can be considered refuse. At present, they are dumped as waste, which result in the loss of enormous mass of organic material.

The agronomic reuse of *P. oceanica* refuse may be an interesting way to provide high-quality organic matter to soils. The dead sheets of *P. oceanica* were traditionally used as compost by the farmers of the coasts Mediterranean [41]. This sea plant is known by its high content in C, N, and P. The desalination of *P. oceanica* did not present a technical problem since *P. oceanica* was a plant with a smooth surface, impermeable to salt existing in its natural environment and a simple rinsing eliminates the quasi totality of chlorides.

It is estimated that the quantity of composted vegetable residues and market wastes produced by Tunis City is greater than 17 t/d, which is a sizable volume of substrate for a biological treatment [42]. Nevertheless more than 90% of vegetable residues (VRs) are dense material with low water content that requires mixing with other wastes or bulking materials.

In this project, we studied the biofungicide effect of VPC against Forl. We analysed the direct effect of VPC on mycelial growth of Forl *in vitro*. The inhibitory effect of VPC was verified in container experiments using sterile soil inoculated with Forl and amended with three rates of VPC. Isolates from VPC were used in order to verify their possible antagonism effect against Forl.

2. Materials and Methods

2.1. Composting Materials

2.1.1. Composting Materials. The initial compost material consisted of 70% of vegetable residues (VR), and 30% of *P. oceanica* residues (PoRs). The materials were stacked in

an uncovered pile and followed by a composting cycle as described by [3].

2.1.2. On Site Sampling. The windrow was sampled during each turning. Four samples were taken at the start of the composting process and samples were collected every 5 days for 150 days. Samples of 5 kg taken from various composts were subdivided into three subsamples introduced by [43]. The first subsample was stored at −20°C for enzyme analysis; the second was used for the physicochemical analyses, and the third was used for microbiological analyses. The subsample for physicochemical analysis was dried at 70°C for 2 days and crushed.

2.1.3. Chemical Analysis. Each fraction obtained was characterised by measuring the following parameters: CO_2 released, pH, Kjeldahl N and inorganic N concentrations. Oxidable-C was determined by dichromate oxidation according to the procedure described in norm NF T 90–101 (October, 1998). Total organic N was measured using the Kjeldahl procedure and the inorganic N content was determined in a 1 mol/L KCl extract (1 : 10, W/V) by steam distillation in the presence of MgO (NH_4+-N) or MgO + Devarda's alloy (NH_4 +-N + NO_2-N + NO_3-N) [44].

The CO_2 evolution was measured according to the incubation method of [45]. Previously screened sample (25 g) at 60% (*w*/*w*) moisture content was sealed in 0.5 L respirometer flasks along with a beaker containing 5 mL of 0.5 mol/L NaOH solution. The samples were incubated at room temperature (25 ± 2°C). During the incubation, the released CO_2 was captured by the NaOH solution, which was then analyzed titrimetrically with 0.2 mol/L. HCl in an excess of $BaCl_2$ at regular intervals.

2.1.4. Evaluation of the Compost Toxicity Using Seed Germination and Root Elongation (GI Index). Germination tests were performed with wheat (Karim, var) provided by the gene bank of National Agronomic Institute Tunisia. Eight seeds, three replicates for each sample of the compost, were left to germinate in the water extract of the compost at 25°C for 72 h. The germination index (GI) was computed by the formula [46]

$$GI = n\text{VSS} \times \frac{\text{RLS}}{n\text{VSC}} \times \text{RLC} \times 100\%, \quad (1)$$

where, *n*VSS and *n*VSC express the number of viable seeds in the sample and in the control, respectively (extract compost was replaced by distilled water); RLS and RLC expressed the root length in the sample and in the control, respectively.

VPC samples used in studies on biocontrol of *Fusarium* were kept at −20°C until use.

2.2. In Vitro Antagonistic Activity of VPC

2.2.1. Effect of VPC on Mycelial Growth of Forl. To study the activity of VPC against mycelial growth of Forl, the potato dextrose agar PDA medium was autoclaved for 15 min at 100 kPa. Then, different concentrations (0.5, 1, 2, 4, 6, 8, 10, 15, and 20%) of VPC (sterilized or not) were incorporated in the potato dextrose agar (PDA) medium. VPC was sterilized during 1 hour at 100 kPa.

The production of Antifungal Volatile (AFV) compounds, by compost were assayed by the sealed plate method as described by [47]. From each compost a 200 μL suspension was spread on trypticase soy agar (TSA, Difco Laboratories, Detroit, M) medium. After incubation at 37°C, a second Petri dish (containing PDA) was inoculated with 6 mm plug of the test fungus in the centre of the plate, inverted and placed over the compost culture. The two plates were sealed together with parafilm (Pechiney Parafilm M PM996 SKU: PH-LF) and further incubated at 25°C. This ensured that the both (compost and Forl) were growing in the same atmosphere though physically separated. As a control, a Petri dish containing TSA medium without compost extract was placed over a plate containing a culture of Forl. Fungal growth was measured as increases in colony diameter of the test fungus at 24 h intervals for a period of 5 days. Each test was replicated 3 times.

The cyanide production was detected using the assay method of [48], where 10% TSA containing 4.4 g glycine liter 1 was inoculated with the compost extract (0.5, 1, 2, 4, 6, 8, 10, 15 and 20%). The lid of each Petri dish contained filter paper impregnated with a picric acid solution (0.5% picric acid and 2% sodium carbonate) was sealed to the bottom Petri dish with Parafilm (Pechiney Parafilm M PM996), and incubated at 28°C for 3 to 5 days. A change in color from yellow to orange-brown of the impregnated filter paper indicated cyanide production.

For detection of antibiotic production, suspensions of VPC were incubated for 60 h in an incubator shaker maintained at 30°C and 170 rpm. The compost extract was centrifuged at 10000 × *g* at 4°C for 10 min. Each supernatant was filtered through a sterile 0.45 μm filter membrane. The cell filtrates were assayed for their ability to inhibit mycelia growth of *F. oxysporum* strain Fo2 by using an agar well diffusion method [49] (Tagg and McGiven, 1971). Molten PDA kept at 45°C was seeded with conidia of *F. oxysporum* and a 5 mm portion spread uniformly over nutrient agar medium (NA, Oxoid). After the seeded layer solidified, three wells were aseptically made using a cork borer, and filled with 100 μL of the test filtrate. The control consisted of 50 μL filter, sterilized distilled water. The samples were allowed to diffuse into the agar and the plate was inverted and incubated at 28°C for 24 h. The plates were examined for halos of inhibition around the wells [49].

2.2.2. Isolation of Bacteria from VPC. For isolation of bacterial strains, 10 g of VPC was suspended in 90 mL of sterile distilled water and shaken for 10 min at 250 rpm. One millilitre of this suspension was used to prepare serial 10-fold dilutions in 0.9% NaCl. Aliquots (100 μL) of an extract of each suspension were spread on Lauria-Bertani agar (LBA: tryptone: 10 g, yeast extract: 5 g, NaCl: 5 g, agar: 15 g and distilled water: 1 L). Representative colonies, that differed morphologically, were selected from the countable plates and restreaked on a new plate of the same media to obtain pure

colonies. Bacterial isolates were stored in 30% glycerol at −20°C.

2.2.3. In Vitro Antagonistic Activity of Bacteria Isolated from VPC In Vitro. Antagonism tests were performed on Potato Dextrose Agar (PDA) in 10 cm Petri plates using a dual culture technique [50]. Bacterial isolates were streaked across the center of the plate, with a second streak made at right angles to the first. Four discs (5 mm in diameter) cut from the edge of 7-day-old cultures of Forl were placed at each side of the antagonist. The distance between the two microorganisms was 2.5 cm. Plates were then incubated at 25°C for one week. Percent growth inhibition of Forl was calculated by the method of [51]. The following formula was used: (R1-R2) R1∗100, where R1 is the farthest radial distance (measured in mm) grown by Forl, after 7 days of incubation, in the direction of the antagonist (the control value), and R2 is the distance of fungal growth from the point of inoculation to the colony margin in the direction of the antagonist. Three replications of each treatment were used and the experiment was conducted for a week.

Antibiotic Substances Produced by Bacteria Isolated from VPC. Bacterial isolates were streaked on TSB medium and then incubated at 30°C for 24 h. A loop of inoculum from a 12 h culture was introduced into 100 mL of the production medium as per [52]. (consisting of 20 g dextrose, 5 g DL-glutamic acid, 1.02 g Mg SO_4, $7H_2O$; 1.0 g K_2HPO_4; 1.5 g KCl and 1 mL of trace element solution (0.5 g $MnSO_4$. H_2O; 0.16 g $CuSO_4$. $5H_2O$ and 0.015 g $FeSO_4$, $7H_2O$ in 100 mL of water). The pH of the medium was adjusted to 6.0–6.2 with 5 N NaOH. The inoculated media were then incubated for 60 h in an incubator shaker maintained at 30°C and 170 rpm. The bacterial suspensions were centrifuged at 10.000 g for 10 min at 4°C. The supernatants were filtered through sterile 0.45 μm disc filters. The cell filtrates were assayed for their ability to inhibit mycelial growth of Forl strain Fo2 by using an agar well-diffusion method [49]. Five mL of molten potato dextrose agar kept at 45°C were seeded with conidia of Forl and spread uniformly over the NA medium. After the solidification of the seeded layer, 3 wells were made using a cork borer and filled with 100 μL of the test fluid. The control consisted of 50 μL of filter sterilized distilled water. The samples were allowed to diffuse into the agar, and the plate was inverted and incubated at 28°C for 24 h. The plates were examined for halos of inhibition around the wells.

Identification of the Potential Antagonistic Bacteria. The identification of bacterial strains was achieved by sequencing the 16S rRNA gene (rrs). Amplification was carried out by PCR with primers F667-pA-rrs AGAGTTTGATCCTGGCTCAG and F668-pH-rrs AAGGAGGTGATCCAGCCGCA designed by [53]. Standard PCR conditions were 1 min DNA denaturation at 94°C, 1 min annealing at 57°C, and 1 min extension at 72°C for 35 cycles. The 16S rDNA sequences were compared with sequences in the GenBank database with the Basic Alignment Search Tool [54].

Effect of VPC in Suppressing the Fusarium Attack in Container Experiments. An agricultural Soil Vertic Xero Fluvent (clay: 27%, silt: 62%, sand: 11%, $pH_{[water]}$: 7, C (0.87%), N: 0.095% C/N: 9.15). was obtained from a field located in the region of Mornag (southwest of Tunis). The soil was moistened with distilled water to 60% of its water-holding capacity and autoclaved for 1 h at 121°C twice, on 2 successive days. The soil was maintained at 60% of its water-holding capacity for a week under sterile conditions. The soil was again autoclaved twice for 20 min at 121°C. After cooling, it was placed in plastic pots (dimensions: 10 cm × 10 cm × 2.5 cm).

This part of experience is divided in two parts: the first one consists of testing the effect of VPC on the fusariose development. The second part concerns the effect of bacteria isolated from VPC on the studied disease. To study the activity of VPC against the development of mycelial of Forl, under pot experience. Different concentrations (0, 10, and 20%) of VPC (sterilized or not) were incorporated and homogenised in the soil. VPC was sterilized during 1 hour at 100 kPa.

Tomato seeds (*Lycopersicuonm esculentum* cv *chourouk*) were surface-sterilized by immersion in 2.5% sodium hypochlorite for 2-3 min and washed thoroughly in 3 changes of sterile distilled water. The seeds were pre-germinated for three days in Petri dishes containing sterile distilled water and the seeds were transplanted into plastic pots (250 cm^3, 4 seeds per pot, and 3 pots per treatment) containing Forl-inoculated soil mixture (positive control). Negative controls were grown in the uninoculated soil mixture.

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The second part of the pot experience was undertaken in the same pot condition as in the first experience at the difference the seeds were inoculated with bacteria isolated from VPC by using a liquid suspension (≈approximately 10^7 colony-forming units (CFU) mL^{-1} of the appropriate isolate), at the rate of 2 mL bacterial suspension per seedling at sowing time. The plants were maintained in greenhouse conditions at 25 ± 5°C and 60 to 90% relative humidity for a period of 6 weeks. Plants were watered as needed and fertilized weekly with 100 mL of Hoagland's nutrient solution. Three replications of each treatment were provided. All values given are averages of three samples for each treatment.

Disease Assessment and Data Analysis. Disease incidence was assessed at 6 weeks by counting number of healthy plants. *Forl* was reisolated from wilted plants by plating stem pieces from the crown region onto PDA. All treatments were replicated in completely randomised blocks using three replications. Analysis of variance was carried out using SPSS software (SPSS for Windows, version 10; SPSS Inc., Chicago, IL, USA), and means were separated by the least

significant difference according to the Student-Newman-Keuls test. Dendrogram were prepared using cluster analysis and average linkage between groups. Sampling unit for statistical analysis is the pot, not the plant.

3. Results

3.1. Stability and Maturity Indexes of VPC. The carbon-to-nitrogen ratio ranged from 30 at the beginning of composting and decreased notably through the process to reach values around 12 (Figure 1). Dehydrogenase activity reached 6 mg TPF g^{-1} DM during the thermophilic phase of the composting cycle. At the end of composting (maturity phase), the values observed were negligible indicating a high degree of maturity. *Salmonella* was isolated only at the beginning of composting. After 40 days, these bacteria were not detected. The mature compost was relatively rich in N (13.0 g kg^{-1}), P (9.48 g kg^{-1}) and Mg (15.80 g kg^{-1}). At the end of composting cycle we obtained mature stable compost; The chemical characteristics of the mature compost expressed in g kg^{-1} are as follow: N: 13, P: 9.48, K: 7.48, Ca: 37.14 and Mg: 15.18.

3.2. In Vitro Effect of VPC against Forl. The incorporation of VPC in the culture medium revealed potent antifungal activity against Forl and complete inhibition of mycelium growth at all the tested concentrations of unsterilized compost extract (Figure 2(a)). However, for sterilized compost extract, only the highest concentrations (15 and 20%) prevented mycelial growth.

3.2.1. In-Vitro Anti-Fungal Volatile (AFV) Produced by VPC Against Mycelium Growth. Different rates of VPC produced AFV's able to inhibit mycelial growth (Figure 2(b)). AFV's became more inhibitory at higher concentrations of VPC. However, we showed that there was no significant difference in inhibition when we concentrated compost over 6% VPC in the media suspension (Figure 2(b)). Cyanide was produced by compost extracts over 2% concentrations. Antibiotics were detected at the lowest concentrations. At 0.5% VPC, the compost extract was able to limit fungal growth (wells method AWDM) (Table 1).

In Vitro Effect of the Indigenous Bacterial Strains of VPC. Nine bacterial strains were isolated from VPC that exhibited antifungal activity towards Forl in agar well-diffusion assays and in dual culture (Table 2). Based on 16S rRNA sequences analysis, the strains B6, B10, B12, BS2, and B17, were identified as *Bacillus sphaericus,* BuC16 as *Burkholderia gladioli*, PPS7 as *Pseudomonas putida,* and the other isolates, BS1 and BS3, were associated with the genus *Bacillus*.

3.3. Suppression of the Fusarium Attack by VPC in Pot Experiments. VPC application on *Fusarium* infected seeds showed that Forl incidence was reduced by unsterilized VPC. The percentage of tomato diseased plants was reduced by both concentrations of unsterilized VPC (10 and 20%) (Figure 3) and suppressive effects were observed against Forl. However, the highest concentration (20% VPC) reduced more the percentage of infected tomato seeds compared with the lower concentrations. This could be related to the strong concentration of nitrate in the 20% VPC. On the other hand, the sterilisation of compost suppress natural microflora able to limit Fo2 strain growth. However, a low quantity of sterilized VPC was showed to slightly reduce the percentage of infected tomato compared to 10 and 20%.

3.4. Effect of Indigenous Bacteria in Container Experiments. Inoculation of seeds with the nine bacterial isolates significantly increased the percentage of healthy plants (Table 2). The percentage of healthy plants ranged from 33 to 96.8%. The best disease control was obtained with isolate B4, B5, B17, and B18, that reduced the wilt incidence to less than 20%. These strains were grouped in the same portion of the dendrogram established by cluster analysis. Cluster analysis determined that the bacteria fell into three phylogenetic groups (Figure 4). In the first group (top of the dendrogram), point B6 and B17, tomatoes treated with this group (GI) were able to reach a percentage of healthy plants ranging between 54.4 and 70%.

Strain *B. sphaericus* BS2 showed the highest level of protection and were grouped with the control test GII, (treatment without pathogenic fungi). Indeed, 96.8% of plants treated with this strain were healthy. The third group GIII (in the centre of dendrogram), composed by B12, PPS7 and Bu C16, was characterized by a percentage of healthy plants ranging between 78 and 83.6%. All the efficient strains studied *in vitro* presented the same trends *in vivo* under greenhouse conditions.

4. Discussion

The VPC can be an interesting biodegradable organic material for compost production. Since both vegetable waste and *Posodonia* pose an ecological problem, their stabilization can help to preserve the environment. Furthermore, the addition of VPC on soil can correct the amount of organic matter on local soils. It is well known that during composting, the majority of fungal pathogens will be eradicated by maintaining a compost temperature above 55°C for 21 days [55]. Moreover, in previous study we showed that during composting, VPC reached a temperature higher than 70°C. This temperature had a selective effect on the microbial community that is of great importance for the suppression in Fusarium wilts [3].

The vast majority of studies on compost suppressiveness demonstrate a relationship between disease suppression and microbial activity. Suppression of Fusarium wilt of tomato using VPC could have been caused by compounds such as cyanide, as well as to some of VPC's indigenous bacterial strains that act as antagonists. After sterilization, VPC lost the ability to suppress *Fusarium*. The inability of sterilized VPC to suppress Fusarium wilt indicates the importance of compost microflora after biological control. Reference [56] showed that compost microflora had no significant effect

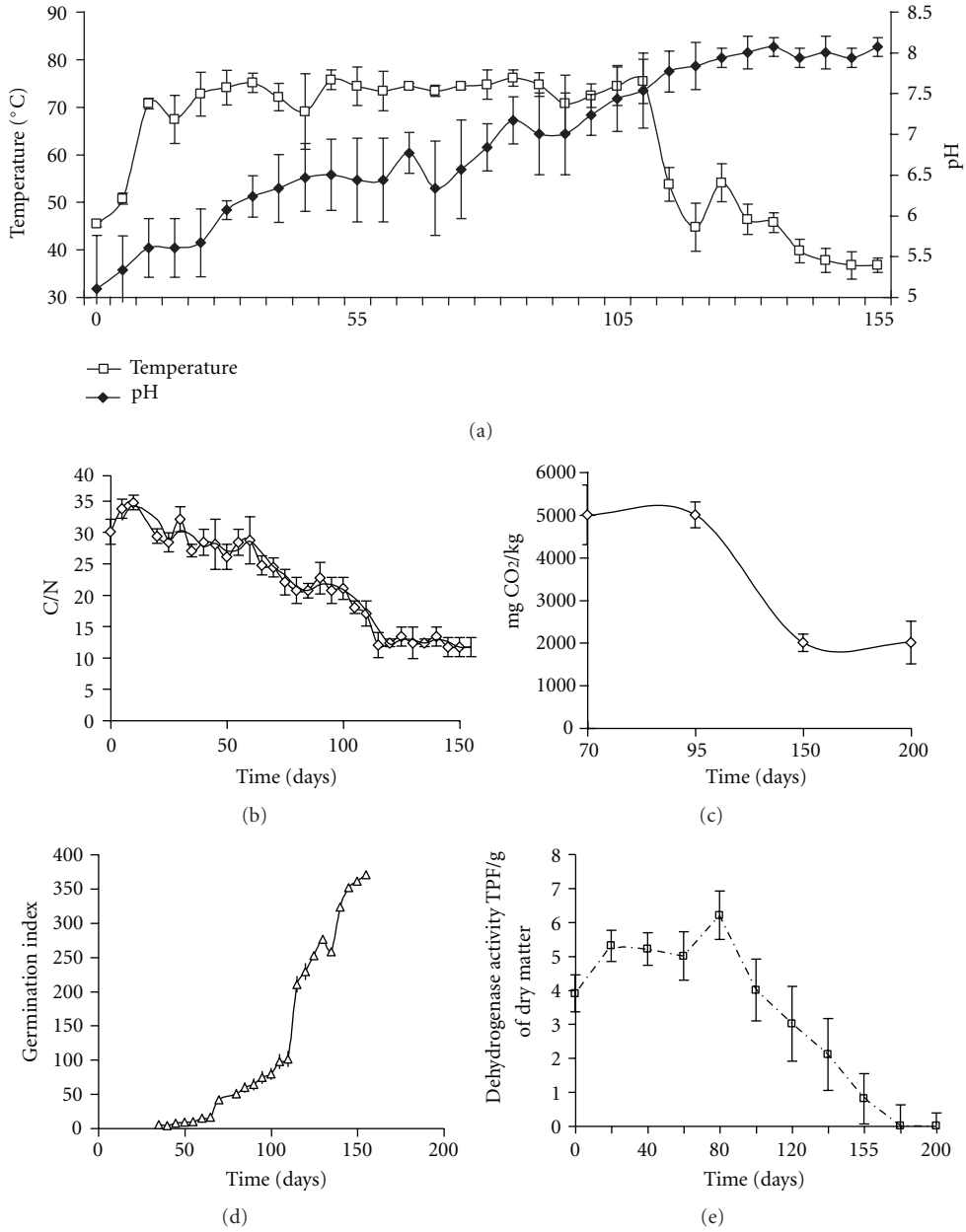

Figure 1: Trends of biochemical and microbiological parameters during composting cycle of mixed vegetable waste (70%) and dead *P. oceanica* (30%) (vol; vol).

on the fungus *Fusarium oxysporum* which could account for the less-efficient suppression of the pathogen with the nonsterilized compost. However, our results were consistent with those obtained by [57], who showed that when the compost was sterilized, it did not control disease, and that compost microflora induced host resistance to the pathogen.

The complete growth inhibition of Forl demonstrated that *Fusarium oxysporum* cannot grow on media containing unsterilized VPC at high rates. This result was not consistent with that reported by [58], who demonstrated that pathogenic fungi were able to grow on solid media containing compost. Total inhibition in our study could be due to effect of Anti-Fungal Volatiles of VPC and/or to the chelation of transition metals by cyanide. [59] reported that cyanide compounds are able to chelate transition metals and also lower the reactivity of metallic iron by forming an inert metal-ligand complex. Chelating of transition metals, such as iron and copper, reduces their bioavailability for fungal growth. Thus the growth inhibition of mycelia could be due to cyanide compounds that can play a critical role in inhibition of fungal growth [60]. Cyanide salts of potassium produce a strong inhibitory effect on the terminal step of the cytochrome-mediated respiratory pathway in fungi. Indeed, [61] showed that the addition of 0.5 mM potassium cyanide reduced the oxygen consumption rate over 70% of biomass content.

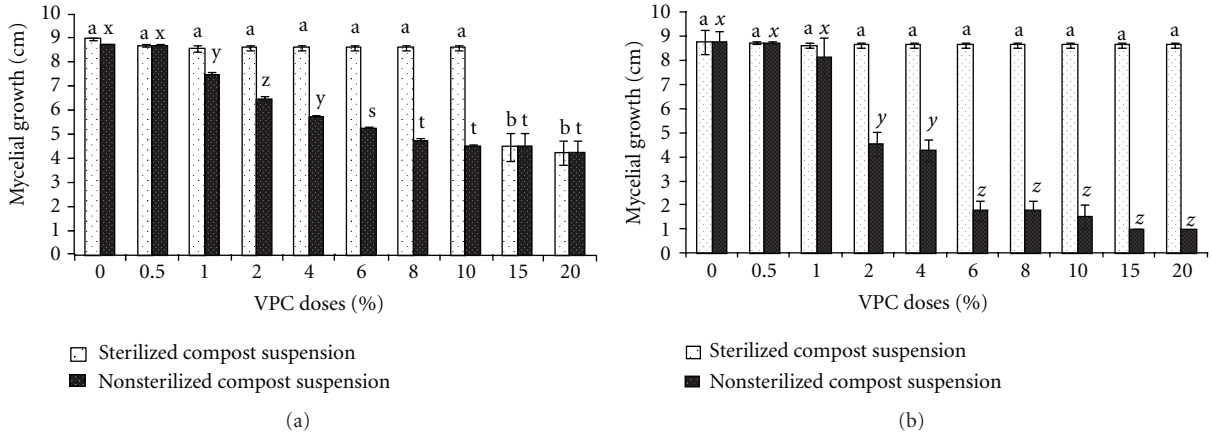

Figure 2: Effect of VPC extract (a) and volatiles produced by VPC extract (b) against Forl on mycelial growth of Forl.

Table 1: Effect of doses compost in volatiles, antibiotic, cyanide production, and growth of fungi in sterilized extract compost and non sterilized compost.

	Compost extract (%)									
	0	0.5	1	2	4	6	8	10	15	20
Volatiles	−	−	−	−	+	+	+	+	+	+
Cyanide	−	−	−	−	+	+	+	+	+	+
Antibiotic	−	+	+	+	+	+	+	+	+	+
Growth of fungi in SEC	−	+	+	+	+	+	+	+	+	+
Growth of fungi in NSEC	−	−	−	−	−	−	−	−	−	−

SEC: sterilized extract compost; NSEC: nonsterilized extract compost (+): positive production, (−): negative production. Cyanide production was detected as described by [48],

Table 2: Effect of Bacillus isolates obtained from the compost materials on *in vitro* growth of Fusarium oxysporum f.sp. radicis-lycopersici and determination of the ability of the most effective bacterial isolates in dual culture on producing cyanide, volatiles and antibiotics.

Isolates	Identify of the selected isolates	Dual culture assay			
		% mycelial inhibition	% fungal inhibition by volatiles	Antibiotic production	Cyanide production
B6	*B. sphaericus*	$42.30^{i} \pm 3$	$8.99^{b} \pm 0.89$	+	−
B10	*B. sphaericus*	$66.33^{g} \pm 0.89$	$52.78^{l} \pm 0.89$	+	+
B12	*B. sphaericus*	$56.36^{k} \pm 0.89$	$23.89^{i} \pm 0.89$	+	−
B17	*B. sphaericus*	$46.80^{l} \pm 0.89$	$11.55^{i} \pm 0.89$	−	+
BuC16	*Burkholderia gladioli*	$41.00^{i} \pm 0.89$	$7.07^{k} \pm 0.89$	−	−
PPS7	*Pseudomonas putida*	$66.33^{g} \pm 0.89$	$44.77^{h} \pm 0.89$	+	+
BS1	*Bacillus*	$7.06^{m} \pm 0.89$	$1.08^{m} \pm 0.89$	+	−
BS2	*B. sphaericus*	$41.00^{i} \pm 0.89$	$6.67^{n} \pm 0.89$	+	−
BS3	*Bacillus*	$15.56^{n} \pm 0.89$	$4.79^{e} \pm 0.89$	+	−

Percent growth inhibition was determined after days of incubation using [51] formula. Values followed by the same letter were not significant ($P \leq 0.05$) by student's Newmans Keuls test. (+) production, (−): nonproduction, ±: indicate standard error of the mean.

Cyanide produced by antagonistic bacteria results in a natural mechanism of plant defence. The forms of cyanide most often discussed from a monitoring viewpoint were free cyanide, weak acid dissolvable (WAD) cyanide, and total free cyanide consisting of HCN and CN. We noted that WAD cyanides were the form required for plant defence. Some authors established that when compost was added to soil, it enhanced the growth of fungi compared to a control (soil not amended with compost) and the WAD cyanide quantities were concentrated in plant shoots [62].

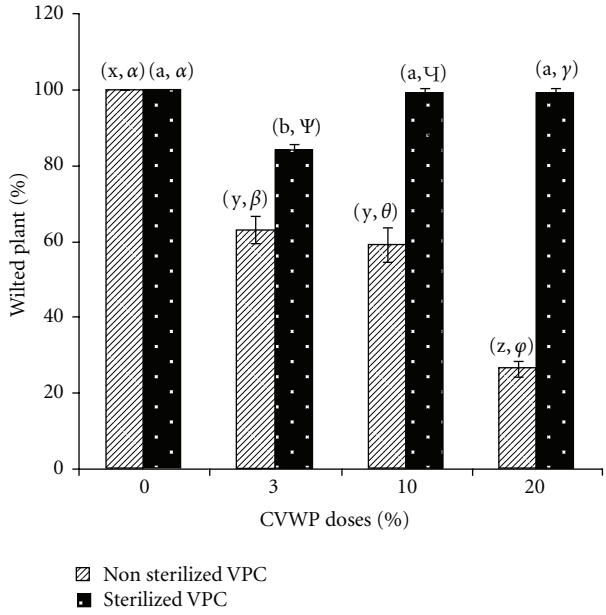

Figure 3: Newman's keuls test differences were assessed at $P < 0.05$. Sampling unit for statistical analysis is the pot, not the plant. (a, b, x, y and z) means the comparison between doses of VPC and (α, β, Ψ, θ, Ч, φ and γ) means differences between sterilized and no sterilized VPC.

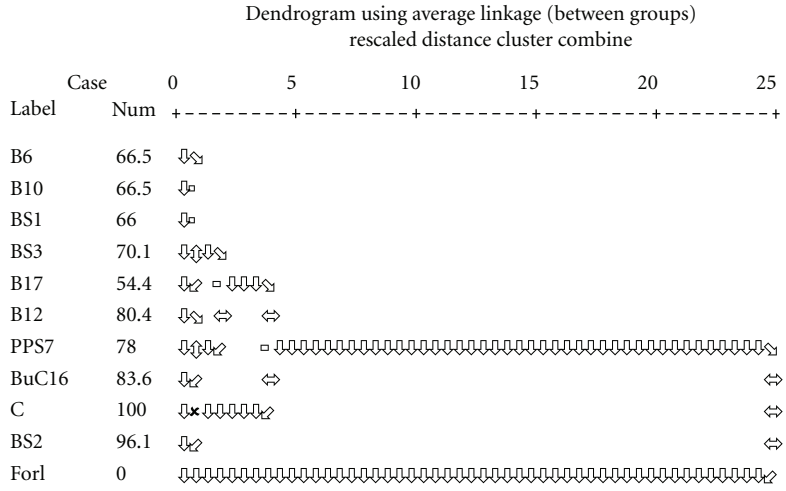

Figure 4: Dendogram grouping of 9 strains isolated from VPC, based on healthy plant percentage. Experience realized *in situ* under green house condition.

The significant reduction of damping-off incidence on tomato plants using the VPC amendment may be attributed to the effect of polyphenols and other chemical compounds. Several researchers have demonstrated that micro-organisms isolated from compost are able to suppress plant disease. Some phytopathogenic bacteria like *Pseudomonas syringae* pv. *savastanoi*, *Corynebacterium michiganense*, and *Xanthomonas campestris* are also inhibited by cyanide present in composts in their original concentration [57].

It is possible that indigenous bacterial strains were also involved in the inhibition of fungal growth. Our study showed that some VPC bacteria play a role against Forl. [63] demonstrated significant disease suppressiveness against *Fusarium* that was induced in the compost-treated soil was mainly attributed to the shift in the soil microbial community. Our results showed that species of *Bacillus*, *Burkholderia,* and *Pseudomonas* isolated from VPC exhibited antimicrobial activity against Forl. These antagonists may have different mechanisms of action including interference with spore germination or inhibition of spore germ tube elongation through abnormal hyphal swelling [64]. They may also be responsible for lysis and complete degradation of the fungal hyphae [65].

The reduction of the Fusarium growth *in vitro* by *Bacillus* and formation of inhibition zones were also reported by [66]. The effect of endogenous compost microflora against Forl

could be due to the antibiotics secreted by bacteria, which may confer fungicidal properties. This finding confirms the hypothesis of [67], who considered that the control of soil-borne plant pathogenic fungi by using organic amendments is due to a specific suppression, which is related to an increase in the population of specific groups of microorganisms that act as antagonists to the plant pathogens.

The inhibition effect of sterilized VPC at high rates may be due to the chelating of nutrients (from the environmental medium) necessary for fungal growth. High nitrogen content could also inhibit the fungal growth. Indeed, Forl was found to be more sensitive to unsterilized VPC. This result seems to be evident due to the antagonistic bacterial effect. In the present study *Burkholderia gladioli* strain BuC16 exhibited strong biocontrol activity and increased the percentage of healthy inoculated plants.

Abbreviations

VR: Vegetable residues
PoR: *P. oceanica* residues
VPC: Vegetable *Posidonia oceanica* compost
MSW: Municipal solid waste
CCR: Control of crown and root rot
AWDM: Agar well diffusion method
PDA: Potato dextrose agar
WAD: Weak acid dissolvable
AFV: Antifungal volatile
t/d: Tons per day
CO_2: Carbon dioxide.

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