Physical and Biological Characteristics of Some Animal Manure Composts and Biological Activity of their Water Extracts Against *Agrobacterium tumefaciens*

**ABSTRACT**

Physical properties, microbial composition and biological characteristics of composted organic materials are important factors to be considered when producing compost. For a successful use of some locally produced composts, a laboratory study was carried out to evaluate some physical and biological properties of nine composts and the efficiency of their extracts was tested *in vitro* against the causal agent of crown gall *Agrobacterium tumefaciens* (strain C58). The physical properties of the composts revealed that they had an alkaline pH (>7.8) and generally a high electrical conductivity (EC), which ranged from 3.08 dS·m⁻¹ (C6) to 8.29 dS·m⁻¹ (C2). Values of carbon-to-nitrogen ratio (C/N), dry and organic matter contents and the dry density of tested composts showed that they were stable and matured products. The biological analyses showed that they contained a higher microbial population based on bacteria and actinomycetes. They were characterized by a weak enzymatic activity (cellulase and protease) and by the absence of phytotoxicity and therefore, ideal to be used softly in horticulture production. The *in vitro* experiment consisted of nine extracts of composts and an antagonistic bacteria K84 as a control, which were arranged in a complete randomized design (CRD) replicated thrice. The result showed that all the extracts tested were effective in reducing *A. tumefaciens* strain C58 development. The best antibacterial activity was recorded by the C7 extract, which showed an inhibition zone of 24.57 mm. No significant difference was found between the extracts and the control K84 after 48 h of incubation.

**Key words:** Physical characteristics, enzymatic activity, microbial population, crown gall, inhibition zone diameter.

**INTRODUCTION**

Composting is a traditional method of waste treatment that means a stabilization of organic matter to be applied to soil (Pascual et al., 2004). It has been defined as intense microbial activity leading to decomposition of most biodegradable materials, usually mixtures of organic materials (Boulter et al., 2000; Weltzien, 1991). The process is considered to be the most efficient treatment in producing an environmentally safe and agronomically advantageous soil organic amendment of acceptable operational costs.

The microbial community in compost converts degradable organic matter into more stable, humified forms and inorganic products and releases heat as metabolic waste product (Ciavatta et al., 1993). Composts attract research interests for their contribution both to recycling waste (Mustin, 1987) and reducing usage of non-
renewable resources such as peat (Kerkeni et al., 2009). Composts are usually free of plant and human pathogens and weed seeds and without odor (Ryckeboer et al., 2002). They are widely used in soils as organic amendments (McClintock and Diop, 2005; Palanivell et al., 2013), but recently, they have shown new properties that increased their value. In fact, suppression of plant diseases with composts was demonstrated in several studies (Pascual et al., 2004; Noble and Coventry, 2005; Kerkeni et al., 2007a, b). Abbasi et al. (2002) showed that the application of animal manure composts led to an increase of biocontrol agents in the rhizosphere and reduced the severity of soil-borne diseases of tomato. The suppressive disease characteristics of composts are attributed to a combination of Physico-chemical properties and biologic attributes (Hoitink et al., 1997; Lozano et al., 2009; Palanivell et al., 2013). In fact, heat treatment of composts generally results in a loss of disease suppressiveness, indicating that the mechanism is often or predominantly due to biological causes (Hoitink et al., 1997). The use of compost could be an interesting solution to cope with soil fertility problems and reduce plant pathogenic diseases. As a matter of fact, the quality of available compost materials can vary widely due to the diverse nature of feed stock and composting processes (Castano et al., 2013; Lozano et al., 2009; Trillas et al., 2006). The successful use of compost whether, as soil amendment or in plant protection, relies on its high quality, preceded by its evaluation, to meet the desired objectives.

This study was therefore conducted to investigate the physical and biological characteristics of some animal manure composts and to test the efficacy of their water extracts on Agrobacterium tumefaciens under in vitro conditions.

### MATERIALS AND METHODS

#### Composts

Nine 12 month-old composts, tagged as C₁, C₂, C₃, C₄, C₅, C₆, C₇, C₈ and C₉ were produced in the composting unit of the Technical Center of Organic Agriculture of Chott-Mariem (Tunisia), according to an aerobic process (Znaidi, 2002), using different kinds of animal manures (poultry, sheep, cattle and horse manures), some vegetable wastes and ground straw. Table 1 show the composition of composts.

<table>
<thead>
<tr>
<th>Composts</th>
<th>Composition</th>
</tr>
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<tbody>
<tr>
<td>C₁</td>
<td>50% CM+25% SM+25% PM</td>
</tr>
<tr>
<td>C₂</td>
<td>60% CM+30% SM+10% ground straw</td>
</tr>
<tr>
<td>C₃</td>
<td>50% CM+25% SM+25% HM</td>
</tr>
<tr>
<td>C₄</td>
<td>50% CM+20% SM+20% PM+10% ground straw</td>
</tr>
<tr>
<td>C₅</td>
<td>25% CM+25% SM+25% PM+25% HM</td>
</tr>
<tr>
<td>C₆</td>
<td>30% CM+30% SM+30% PM+10% ground straw</td>
</tr>
<tr>
<td>C₇</td>
<td>40% CM+40% SM+20% vegetable wastes</td>
</tr>
<tr>
<td>C₈</td>
<td>25% CM+25% SM+25% PM+15% HM+10% ground straw</td>
</tr>
<tr>
<td>C₉</td>
<td>25% CM+25% SM+25% PM+25% HM</td>
</tr>
</tbody>
</table>

#### Physical properties of the composts

Composite samples from each compost were separately collected, oven-dried and ground with a ball mill prior to analysis. They were evaluated for pH, electrical conductivity (EC), percent moisture (%), bulk density and C/N (carbon/nitrogen) ratio. The bulk density was calculated by dividing the weight of the compost sample by its volume in the used container. The pH and EC were determined from a 1:5 (vol/vol) mixture of distilled water and compost; pH was measured using pH meter (model Mettler-Toledo MP225) and EC with a conductimeter (model Amel 123). Organic matter (OM) was obtained by combustion at 900°C for 2 h and C was estimated by the formula C=OM/1.72 (Reddy et al., 2005). N content was determined using the Kjeldahl method (Black et al., 1979).

#### Biological properties

#### Microbial population

Different groups of micro-organisms (bacteria and fungi, actinomycetes and Trichoderma spp.) in composts were determined using selective culture media and the serial dilution method. A sample of 10 g of fresh compost was diluted into 250 ml bottle containing 90 ml of sterile deionized water and stirred for one hour at 200 rpm. After being shaken, serial dilutions were prepared into tubes. Four different media were used for plating 100 μl aliquots of serial diluted suspensions on Petri plates (9 cm diameter). A suspension up to 10⁻³ was plated on media selective for Trichoderma (Williams et al., 2003). A suspension up to 10⁻⁵ was plated on a medium selective for actinomycetes (water yeast agar medium) (McQuilken et al., 1994). A suspension up to 10⁻³ was plated on potato
dextrose agar (FDA, Sigma) for isolation of fungi and a suspension up to $10^{-7}$ plated on Glutamate-Manitol (MG) medium for isolation of the bacteria. Three plates of each medium were inoculated for each dilution. Plates were incubated at room temperature (20 to 25°C) for an adequate period (4 to 5 days) before counting the colony forming unit (CFU).

**Microbial activity**

Microbial activity was assessed by fluorescein diacetate (FDA) hydrolysis method proposed by Inbar et al. (1991) and Adam and Duncan (2001). The method consists of adding 1 g of compost sample to 20 ml of potassium phosphate buffer at pH 7.6. The enzymatic hydrolysis started by adding 200 µl FDA solution (2 mg ml$^{-1}$). The sample was shaken for 20 min at 90 rpm. Hydrolysis reaction was terminated by adding 20 ml acetone. Following filtration, extraction and dilution of 2 ml aliquot for five times, the absorbance was measured by spectrophotometry at 490 nm.

FDA hydrolysis was performed according to Adam and Duncan (2001), following the addition of 6 g of compost sample to 60 ml of 60 mM potassium phosphate buffer at pH 7.6. The enzymatic hydrolysis started by adding 0.4 ml FDA solution (1 mg ml$^{-1}$). The sample was shaken for 10 min in an orbital incubator; 15 ml sub-samples were obtained at 0 and 10 min and the hydrolysis reaction was terminated by adding 15 ml CHCl$_3$/CH$_3$OH (2:1 vol:vol$^{-1}$). Following centrifugation (700×g) and filtration of the aqueous phase, the absorbance of filtrates was measured at 490 nm. Blanks, without addition of FDA, were also included to correct for background absorbance, and the concentration of fluorescein release was determined against a calibration curve, produced by 0-, 1-, 2-, 3-, 4- and 5-µg ml$^{-1}$ fluorescein standards.

**Enzymatic activity of composts**

Cellulase and protease activity was analyzed by methods proposed by Alef and Nannipieri (1995). Cellulase activity was estimated by incubating compost samples (1 g) with carboxymethylcellulose (CMC) sodium salt (0.7%) and acetate buffer for 24 h at 50°C and measuring released reducing sugars in a spectrophotometer at 690 nm. Protease activity was measured by incubating compost samples (0.5 g fw) with sodium caseinate (8%) for 2 h at 50°C using Folin-Ciocalteu reagent and quantifying the released amino acids in a spectrophotometer at 700 nm.

**Water compost extracts**

Water extracts were prepared from composts by suspending composts in tap water (1:5, v/v) in 20 L plastic container and stirring the mixture daily for about 10 min during an extraction period of 5 days (Weltzien, 1992). After the incubation period, the mixtures were filtered through cheese cloth (250 µm) and the obtained extracts were stored at 4°C. They were taken out 30 min before use.

**Agrobacterium rhizogens K84 and Agrobacterium tumefaciens strain C58**

The strain C58 of A. tumefaciens and the reference antagonistic strain K84, used as control, were provided by the olive institute of Sfax (Tunisia). They were sustained on MG medium at 25°C.

**Effect of compost extracts on Agrobacterium tumefaciens (strain 58) development**

The antibacterial activity of each extract against A. tumefaciens strain C58 was tested through the double layer method (Rhouma et al, 2008). Test consists of suspending individually 10 µl of each extract on MG
medium in Petri plates and incubating them for 24 and 48 h at 27°C. On the same day of extract incubation, A. tumefaciens strain was streaked over the solidified surface of MG medium. After incubation, the plates were cleaned with alcohol (70%) and then exposed to chloroform vapor for 30 min under laminar flow cabinet. After evaporation, one ml suspension of the pathogen (108 CFU ml⁻¹) was mixed with 3 ml of LBA (0.6% agar) at 45°C and quickly overlaid to plates containing the extracts. Plates were incubated again at 27°C and checked after 24 to 48 h for the appearance of inhibition haloes surrounding the extracts’ spots. Control plates were represented by the antagonistic bacteria K84. The experiment was carried out in a completely randomized design (CRD) with three replicates and repeated twice.

Statistical analyses

Data were subjected to analysis of variance (ANOVA) with the SPSS software (version 13). Significance of mean differences was determined using the Duncan’s test and responses were judged significant at 5% level (P=0.05).

RESULTS AND DISCUSSION

Physical properties of composts

Table 2 shows the physical characteristics of the composts used in this study. Results showed that moisture content values ranged between 18.5% for C₆ (30% CM+30% SM+30% PM+10% ground straw) and 49.5% for C₁ (50% CM+25% SM+25% PM). Statistically, there is no difference between C₂, C₄, C₉ and C₁ and between C₅, C₆, C₃ and C₈. Mustin (1987) explained that during the thermophilic stage of composting process, composts lose a lot of water and dry up. According to Sullivan and Costello (2010), moisture content of matured compost should be between 35 and 60%, respectively whereas, Fuchs et al. (2001) reported that a good compost should have a moisture content less than 50%. Compost which is too dry (>40%) can be dusty and irritating to work with while compost which is excessively wet (>60%) can be heavy and difficult to uniformly apply (Sullivan and Costello, 2010). C₆ and C₇ exceptionally showed less than 35 moisture percent, all the composts analyzed have a moderate moisture, which allows them to be easily applied as organic amendement and also to induce suppression against soil borne pathogens (Hoitink et al., 1997).

Regarding OM content, values ranged from 22 to 32.2% for different composts types (Table 2). The lowest value of total organic matter (22%) was found for the most dried compost (C₆) and the highest value (32.2%) was found for C₃. It was stated that high quality composts should have a minimum of 50% organic content (Fuchs et al., 2001). Hence, OM content of all the used composts was low. This may be due firstly, to their old age (1 year) and high mineralization degree and secondly, the nature of feed stock used (animal manure) which contained little lignin and cellulose. Larbi (2006) revealed that for young composts (8 to 9 weeks), OM content can reach 62%, while for old composts (aged> 40 weeks), OM content is only 17%.

According to the Table 2, all the studied composts have high pH values which is explained by the nature of feed stocks used (animal manures are frequently alkaline (Mustin, 1987). The pH values ranged between 7.8 and 8.52. The highest pH was recorded in both composts C₆ and C₇ while the lowest value was enregistered in composts C₄, C₁ and C₉. This finding confirms the studies of Gondek et al. (2014) indicating that the composting process leads to an increase of substrates pH following the loss of hydrogen and water during microbial activity (McClintock et al., 2005; Noble and Coventry, 2005; Sanchez-Monedero et al., 2001).

According to Boulter et al. (2000), the pH value settles between 8.1 to 8.6 as the compost stabilizes and these pH favours the development of actinomycetes and alkaline bacteria. Composts with pH>5 were shown to increase bacterial biocontrol agents (Hoitink et al., 1997). The EC values ranged from 2.95 to 8.29 dS m⁻¹ for different compost types. The highest value of EC (8.29 dS m⁻¹) was found for C₂ (60% CM+30% SM+10% ground straw) and the lowest value of EC (2.95 dS m⁻¹) was determined for the C₀ composed of 25% CM, 25% SM, 25% PM and 25% HM. The elevated value of electrolytic conductivity in the composts could have resulted from the mineralization of

Table 2: Physical properties of the composts used in the study. Each value is the overage of three repetitions. Different letters within line represent values that are significantly different at P=0.05 based on ANOVA and Duncan test.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C₁</th>
<th>C₂</th>
<th>C₃</th>
<th>C₄</th>
<th>C₅</th>
<th>C₆</th>
<th>C₇</th>
<th>C₈</th>
<th>C₉</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>50.50a</td>
<td>58.25bcd</td>
<td>60.75bc</td>
<td>55.00cd</td>
<td>65.00b</td>
<td>81.50a</td>
<td>67.25b</td>
<td>63.00bc</td>
<td>59.00bcd</td>
</tr>
<tr>
<td>MO (%DW)</td>
<td>32.00a</td>
<td>29.40bc</td>
<td>32.2a</td>
<td>26.00c</td>
<td>31.50a</td>
<td>22.00d</td>
<td>23.50cd</td>
<td>26.50c</td>
<td>25.50c</td>
</tr>
<tr>
<td>pH</td>
<td>7.87ed</td>
<td>8.21b</td>
<td>8.11c</td>
<td>7.80d</td>
<td>7.92d</td>
<td>8.52a</td>
<td>8.26b</td>
<td>7.80e</td>
<td>8.50a</td>
</tr>
<tr>
<td>CE (dSm⁻¹)</td>
<td>5.56c</td>
<td>8.29a</td>
<td>4.80g</td>
<td>4.36i</td>
<td>5.22d</td>
<td>3.08b</td>
<td>3.77f</td>
<td>5.95b</td>
<td>2.95f</td>
</tr>
<tr>
<td>C/N</td>
<td>12.32c</td>
<td>11.87c</td>
<td>10.17d</td>
<td>9.39d</td>
<td>19.91s</td>
<td>14.87b</td>
<td>10.76cd</td>
<td>12.2c</td>
<td>15.2b</td>
</tr>
<tr>
<td>DS (g.cm⁻³)</td>
<td>0.62d</td>
<td>0.61d</td>
<td>0.58e</td>
<td>0.71b</td>
<td>0.66c</td>
<td>0.80a</td>
<td>0.79a</td>
<td>0.70b</td>
<td>0.61d</td>
</tr>
</tbody>
</table>
organic matter (Gondek et al., 2014) and resulted also from the nature of animal manure used (cattle manure) known for its high salt content. According to Fuchs et al. (2001), compost salinity should not exceed 4 dS.m⁻¹ due to adverse effects on sensitive species. Excess soluble salts in composts applied to soil may distort the balance in the soil solution (Gondek et al., 2014). Moreover, it was demonstrated that composts with high pH and EC values, have high microbial activities (Noble and Coventry, 2005) and that compost pH and EC are decisive factors in a compost’s capacity to act as biocontrol product since their values will decide on the pathogen’s establishment in the soil or not (Pascual et al, 2004).

Table 2 also indicated that C/N ratio ranged from 9.39 to 19.91. The highest value was measured in C₅ while the lowest C/N ratio was found for C₄. The significant difference between the different composts is perhaps due to a variation in their degree of organic matter decomposition. In fact, the C/N ratio is an indicator used to evaluate compost stability and maturity of compost (Gurama et al., 2012; N’Dayegamiye et al., 1997). It was stated that when C/N ratio is less than 20, the compost is matured and can be used without any restrictions (Garcia et al., 1992).

Regarding the bulk density, the results showed that values ranged from 0.58 to 0.8 g. cm⁻³ for different compost types. The highest value of bulk density (0.8 cm⁻³) was found for C₃ and the lowest value of bulk density (0.58 g. cm⁻³) was found for C₆. These values were considered high in comparison to the findings of El-Sayed (2015), who found that bulk density of composts based on cattle manure and plants residues was between 0.42 and 0.65 g. cm⁻³ and between 0.447 and 0.502 g. cm⁻³ (Huerta-Pujol et al., 2010). It could be seen also that the bulk density of compost decreases with increasing compost organic matter. It decreases from 0.8 to 0.58 g. cm⁻³ when the organic matter increased from 22 to 32.2%. This result is in compomity with that of El-Sayed (2015) who showed the relationship between the bulk density and the total organic matter.

### Biological properties

#### Microbial density

The most numerous group of micro-organisms in the analyzed composts were bacteria and actinomycetes, in contrast to fungi, which were the least numerous (Table 3). These results are in compomirty whith the findings of Gondek et al. (2014) and Pugliese et al. (2008). Generally, the composts C₈, C₃ and C₇ showed higher number of micro-organisms. Fungal population were higher in composts C₈, C₅ and C₃ by 36, 33 and 30 × 10⁴ CFU. g⁻¹ respectively, whereas, C₉ and C₄ contained over 8-fold fewer by 4 and 8 × 10⁴ CFU g⁻¹ respectively. The total number of fungi isolated from our composts was relatively low when compared to the number (10⁹) founded by Gondek et al. (2014) and Larbi (2006) in composts aged of 30 weeks and consisting of plant waste and urban biodegradable waste. This is perhaps due to the difference both in the age and in the nature of feed stock used.

Microbial densities in the composts showed that *Trichoderma spp.* concentration varied from 1.5 to 24.5 × 10³ CFU. g⁻¹ (Table 3). The higher concentration (24.5 × 10³ CFU. g⁻¹) was determined in C₉. According to the Table 3, the total fungal population was widely represented by the genus *Trichoderma*, which reached 94.1, 68 and 46.9% of the total fungi in C₉, C₅ and C₇ respectively. The lowest number of *trichoderma spp* was determined in the compost C₈ (1.5 CFU.g⁻¹). The antagonistic activity of the genus *Trichoderma* such as *T. harzianum* and *T. hamatum* was repeatedly shown and compost can be a natural resource for these antagonists (Chung and Hoitink, 1990; Kwok et al., 1987; Pugliese et al., 2008). These fungi were isolated in several studies from different types of composts and compost extracts (Cotxarrera et al., 2002; El-Masry et al., 2002; McQuilken et al., 1994) and identified as agents.
involved in biological control against several fungal diseases. Consequently, the use of the composts C₁, C₂ and C₃ might have an antagonistic effect on plant diseases.

The number of bacteria varied from $0.5 \times 10^7$ CFU·g⁻¹ in C₆ to $6.5 \times 10^7$ CFU·g⁻¹ in C₁. The actinomycetes thrived best in the compost C₃ with $63.5 \times 10^5$ CFU·g⁻¹. The lowest concentration was found in C₁ ($14.7 \times 10^5$ CFU·g⁻¹). These results were relatively lower as compared to results found in young composts. In fact, Larbi (2006) counted values between $10^8$ and $10^9$ CFU·g⁻¹ of bacteria in composts, consisting of various green waste and aged of 8 and 56 weeks respectively. Similar results was also found by Levanon et al. (2002) in a mixture of cattle and chickens compost, and Pugliese et al. (2008) in green, organic domestic wastes and urban sludges. The difference of our results with previous works is surly due to the nature of initial composition and essentially the age of composts (Mustin, 1987). This result confirms that of Gattinger et al. (2004) who stated that microbial biomass in composts declines with time.

### Microbial activity

High rate of FDA was absorbed in C₇ ($0.56 \mu g \cdot g^{-1} \text{ Dry weight. min}^{-1}$) compared to C₀ which had the lowest value of FDA ($0.26 \mu g \cdot g^{-1} \text{ Dry weight. min}^{-1}$) (Table 4). Larbi (2006) showed that microbial activity in a young compost (30 weeks) is higher than $12 \mu g \cdot g^{-1} \text{ dry weight. min}^{-1}$. The low values found in this study were attributed to the advanced age of these composts and their maturity. Gattinger et al. (2004) stated that microbial activity in composts declined also with time. Several studies have shown a positive correlation between the suppressive potential of composts and FDA (Bananomi et al., 2010; Mandelbaum et al., 1990; Noble and Coventry, 2005; Spyridon et al., 2006). According to Boehm et al. (1992) and Gattinger et al. (2004), the estimate of time during which the compost is able to maintain its suppressive capacity could be determined by measuring its FDA hydrolysis.

### Enzymatic activity of composts

According to Piotrowska-Cyplik et al. (2008), a better indicator in an assessment of the biological activity of composted material is their enzymatic activity. Table 4 shows the cellulase activity determined by the amount of reduced sugars after incubation of composts with CMC (carboxymethylcellulose). The results revealed that C₀ and C₃ had the highest cellulase activity by $3746.49$ and $2158.54 \mu g \cdot g^{-1}$, respectively. The compost C₄ showed the lowest value (172.7 $\mu g \cdot g^{-1}$). Despite their equal initial content in plant waste (ground straw (10%), (Table 1), which is considered as a high cellulose source (Chang et al., 2009), their same pH and total OM content (Table 2), the composts C₀ and C₄ showed a significant difference concerning this enzyme. The higher cellulase activity noted in both C₃ and C₆ composts could be correlated to their actinomycetes concentration (Table 3). According to Mustin (1987) and Chang et al. (2009), actinomycetes were considered among the main cellulolytic microorganisms existing in a compost.

According to Table 4, protease activity reached its maximum in C₁ (78.42 $\mu g \cdot g^{-1}$) and C₃ (77.11 $\mu g \cdot g^{-1}$). However, the least value was founded in C₀ (30.58 $\mu g \cdot g^{-1}$). The high concentration of bacteria measured in C₁ and C₃ (Table 3) could probably be the most important responsible for their high protease activity as compared to C₀. According to Valsange et al. (2012) bacteria are very good sources of protease enzymes as compared to animal or plant source.

### Effect of compost extracts on Agrobacterium tumefaciens development

The in vitro essay showed that the nine compost extracts were effective in reducing Agrobacterium tumefaciens strain C58 growth after 24 h (Figure 1). A significant difference was noted across the extracts used. The best antibacterial activity was recorded by the C₁ extract (40% CM+40% SM+20% vegetable wastes), which showed an
inhibition zone of 24.57 mm. The C4 and C5 extracts were the least ones in reducing pathogen development by 6.09 and 18.96 mm respectively as compared to the control K84 (19.5 mm). This variability noted between compost extracts could be attributed to their microbial diversity and diversity of substances liberated from those organic products. After 48 h of incubation, all the composts extracts showed similar suppressive effect than the reference strain K84, but their activity was more improved. Reduction of disease had reached 32% (C1 and C6). This improvement of extract activity is presumably due to the expression of all the micro-organisms contained in the extracts (El-Masry et al., 2002), and the production of higher amount of antibiotics in the culture media (Hoitink et al., 1997).

Micro-organisms and antibacterial substances of compost extracts may require more time to express their optimal biological potential. According to Weltzien (1992), efficacy of compost extracts may vary considerably. This may be, in part, due to differences in procedures used for preparation of the extracts, the source, composition, quality and maturity of the compost, length of storage and possibly other factors. In addition and in previous studies, Penyalver et al. (2001) reported that A. rhizogenes K84, produced iron-binding compounds (hydroxamate iron chelator) in large amounts as compared to A. tumefaciens, when grown in iron-deficient medium (this is the case of the medium used in this study). This product may be identical to a previously described antimicrobial substance called ALS84. According to these results we can attribute a part of the suppressive effect of compost extracts to their iron content. In fact, chemical analyses of used extracts showed in our previous work (kerkeni et al., 2009) that they contained more than 0.3 ppm of iron.

**Conclusion**

This study showed that all the composts used are alcaline (pH>7.8) and present a high electrical conductivity (CE> 3 dS.m⁻¹). Through their measured physical characteristics (C/N, percent, organic matter and bulk density) and biological properties (enzymatic activity). They can be considered as stable and matured products. *In vitro* bioassay, showed also that compost extracts have significant influence on *A. tumefaciens* strain C58 as compared to the antagonistic bacteria K84. This is attributable to favourable environmental conditions created by the compost which favours the proliferation and antagonistic activity of the biocontrol agents present in the compost.

Consequently, the biological parameters seem to be more informative for predicting suppressiveness, than the physical parameters. Further research to isolate and identify the micro-organisms responsible for the antibacterial activity from the most suppressive composts and to use them as effective biocontrol agents, are recommended and their ability to suppress the crown gall disease needs to be tested *in vivo*.

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