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Quality Assessment by *Aspergillus niger* of an Onion, Cattle Manure and Alfalfa Waste Compost Blend

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Using different agricultural waste composts as organic amendments or substrates for seedlings has increased in recent years. From remote times, organic waste has been associated with disease and agriculture fertility. However, determining the load of phytopathogenic microorganisms that can persist in mature compost is essential given the risk of them becoming a transmitter of disease. In the present work, *A. niger* survival in mature compost made with onion waste mixed with cattle manure and alfalfa waste was assessed. The relationships between the inoculum of *A. niger* loads present in mature compost and the quantitative ranges of the evaluated parameters were established. To this end, the *Aspergillus niger* inoculum in the onion compost, obtained after a 6-month composting process, was used. The pH, EC and C:N ratio values in the three compost products do not limit them from being used as substrates in horticultural seedling production or as an organic soil amendment. These results are consistent with the research reported by other authors. Hence this compost can be successfully used as a substrate component in horticultural seedling production and as an organic fertilizer in soils without damaging crops.

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1. INTRODUCTION

One of the most important, national and regional vegetable productions for human consumption is fresh onion. The main health problem for this product is caused by *Aspergillus niger,* a causal agent of a disease known as *cinder*. Its incidence becomes particularly important during the conservation period, and generates losses of stored bulbs that range between 20% and 80%, [1]. Fig. 1 show the appearance of bulbs affected by *Aspergillus niger* after remaining in conservation stacks for 3 months.

When the time comes to commercialize onions, tips and roots are removed and onions are brushed in packing sheds. The aim of these tasks is to eliminate plant remains and any loose external cataphylls which, along with the discarded bulbs, make up the waste from packaging operations [2]. This waste generates serious environmental problems because of its volume and placements. So, recycling it by a composting process is an effective, environment-friendly and feasible alternative from the production viewpoint.

During the degradation process, resident microorganisms in waste use various carbon sources, mainly lignocellulose polymers, which are actually responsible for compost maturation [3]. However, it is vital that excess pathogen loads are degraded and removed in this stage in order to avoid composts becoming disease-spreading agents and, conversely, to become suitable products for agricultural use.

Composts are considered to improve organic soil. In intensive horticulture, using compost as a soil amendment, fertilizer for cultivated plants, and/or a substrate component for seedlings is common place [4-6]. However prior to use, it is essential to determine its quality, which varies depending on the substrate used and/or the processing methodology implemented [7,8].

Christensen et al. [9] evaluated different strategies to describe the health quality of industrial-scale composting. Some authors have reported that, in certain cases, compost applications have lowered the incidence of pathogens like *Phythiumsp., Phytophthora SP., Penicillium sp*. through mechanisms known as "general suppression" [10]. Nevertheless, compost made with onion waste could be infected with *A. niger* if this pathogen is not removed during the composting process. Therefore, it could become a source of inoculum which could affect plant health when applied as an organic soil amendment or as a substrate for horticultural crops [11]. This is why it is most important to determine presence of pathogens, apart from the physical, chemical, and biological properties of compost, because their activities can be dangerous for both humans and animals, and can also affect plant health and/or productivity. Such information optimizes quality standards [11,12].

Composting is the biological conversion of solid organic matter in usable end products [13].

Fig. 1. Appearance of bulbs affected by *Aspergillus niger* after remaining in conservation stacks for 3 months

In the present work, *A. niger* survival in mature compost made with onion waste mixed with cattle manure and alfalfa waste was assessed. Whether the mature compost met the physical, chemical and health characteristics required for agricultural use was also established. Finally, relationships between the inoculum of *A. niger* loads present in mature compost and the quantitative ranges of the evaluated parameters were established.

2. MATERIALS AND METHODS

2.1 Description of the Experimental Site and the Experiments Carried Out

The field experiment was conducted in the research field of the Area of Agriculture, National University of Comahue-CURZA, situated in the city of Viedma, Río Negro (40° 49 'S; 63º 05' W). Three mature composts were used, which were prepared with mixtures of the following material:

- ✓ Onion-Manure-Alfalfa (OMA) in the 1: 0.5: 0.3 proportions.
- ✓ Onion-Manure (OM) in the 1:1 proportion.
- ✓ Onion-Alfalfa (OA) in the 1:0.7 proportion.

Where

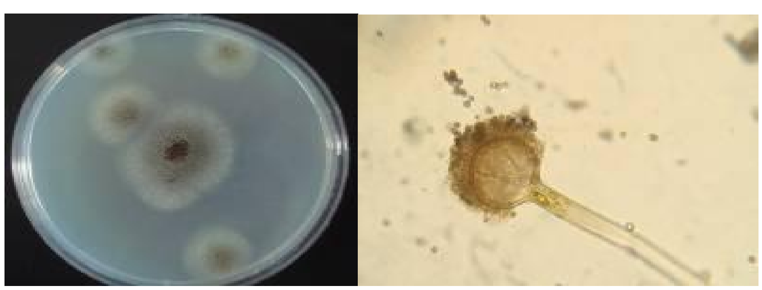
- ✓ Onion waste (O): remains of leaves, stems, roots and cataphylls from packing sheds.
- ✓ Alfalfa waste (A): the waste material from a stretch wrapper alfalfa plant for exports. Alfalfa is used as a natural fertilizer product, which provides many nutritional benefits.
- ✓ Dairy cattle manure (M): manure from cattle is one of the finest materials that can be added to any compost pile.

After 180 days of composting (Fig. 2), one sample of every compost was taken, (1 kg). Samples were sieved with a 10 mm mesh, homogenized and taken to the laboratory to calculate the *A. Niger* population levels and to make physico-chemical determinations.

2.2 Identification and Quantification of *Aspergillus niger*

Three subsamples, (100 g of each compost) were taken to be then homogenized. Next 10 g aliquots of each subsample were taken and diluted in 90 mL of sterile water (10-1). From this dilution, serial dilutions up to 10^{-5} were made. One milliliter of the 10^{-4} and 10^{-5} dilutions of each subsample was sown in Petri dishes with agar potato-dextrose (APD) culture medium, stored in boxes; boxes were incubated at 24°C in the dark for 7 days. Then *A. Niger* colonies (Fig. 3), were counted according to their macroscopic and microscopic char-forming units per gram of substrate dry weight (cfu/g SDW).

Fig. 2. Final compost obtained from onion waste



A B

Fig. 3. A. Characteristic *A. niger* colony grown on APD under the conditions described in the methodology. B. characteristic *A. niger* structure (conidiophore, long head, sterigmata and conidia)

2.3 Statistical Analysis

The variation of the properties of different compost studied was analyzed by ANOVA and Tukey test for comparison of means ($p \leq 0.05$). The INFOSTAT, version 2011 statistical software was used.

3. RESULTS AND DISCUSSION

The composting process serves to transform waste from the onions classified for sale which, after the post-harvest period, become a product of agronomical value. This waste is composed mostly of dehydrated cataphylls, which are shed from bulbs. These tend to be colonized by saprophytic organisms, which remain if the composting process does not remove them.

Atlas and Bartha [14] found that the cellulolytic fungi species most commonly encountered in different composted materials belong to the genera *Aspergillus*, *Fusarium* and *Penicillium* and *Trichoderma.* Cruz et al. [15] assessed two mature composts prepared with gardening and horticultural waste, and the presence of the genus *Aspergillus* was highlighted in both. Below Table 1 presents the *A. niger* population levels in the mature composts of the three mixtures (OMA, OM and OA). CFU were counted at the 10^{-5} dilution.

The largest *A. niger* population was obtained in the OM blend, where the C:N ratio obtained an intermediate value (Fig. 4). Although this ratio in OMA, where the smallest number of cfu was recorded, fell within the appropriate parameters, the total quantities of both nutrients were low (Fig. 4); that is, premature nutrition depletion may occur, or fungal biomass production may slow down. Finally in OA, which obtained the lowest C:N ratio value, the population level was intermediate. As previously stated, this type of ratio does not affect the process, but with loss of nitrogen, it would not be possible to sustain the growth rate of the fungus. These results could infer that there is an optimum C:N ratio for *A. Niger*, and the growth rate of the fungus would lower above or below it.

Significant differences in the fungal load of three substrates were observed (Table 1). The largest *A. Niger* population was found in OM, followed by OA and OMA, and in that order. Previous studies have identified significant differences in *A. Niger* fungal biomass production and its secondary metabolites when grown under the same environmental conditions, but on different substrates [16-18].

Table 1. The *A. niger* population levels in the three mature composts

	OMA	OM	OA
CFU/g SDW	0.11×10^5a	0.44×10^5c	0.22×10^5b

CFU/g SDW = Colony-forming units per gram of substrate dry weight. In the same row, different letters indicate significant differences (p ≤0.05). OMA, onion-manure-alfalfa; OM, onion-manure; OA, onion-alfalfa; p ≤0.05). OMA, onion-cattle manure-alfalfa; OM, onion-cattle manure; OA, onion-alfalfa

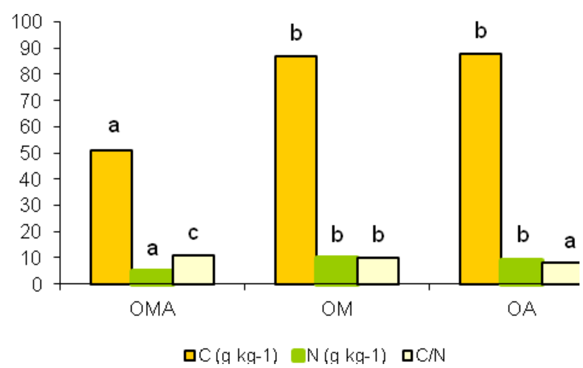


Fig. 4. C content ($g.kg^{-1}$), N content ($g.kg^{-1}$) and the C:N ratio of the studied composts. Different letters on the same colored bars indicate significant differences among treatments (p≤0.05). OMA, onion-cattle manure-alfalfa; OM, onion-cattle manure; OA, onion-alfalfa

Manure is generally a component rich in carbon, and the OM blend presented a higher proportion of this component. Adegunloye et al. [19] used cow manure to provide microorganisms input to compost and reported that the *A. niger* fungus population was the most abundant. With these references, it can be deduced that the largest fungus population in OM implied, among other aspects, that apart from manure being an excellent source of energy, it can also provide an additional inoculum load.

A. Niger is one of the fungi which are best able to degrade lignocellulose materials as it synthesizes large quantities of extracellular hydrolytic and oxidative enzymes. However, this enzyme activity is related to the temperature that the process reaches, mainly because this fungus is considered mesophyll [20]. Mycelial growth reaches critical temperatures at between 5°C and 45°C, with an optimum of 30°C [21]. The temperatures achieved during composting depend, among others, on the materials to be composted. Previous studies have determined that both mesophyll (< 40°C) and thermophilic (> 60°C) temperatures are reached in the OM, OMA and OA mixtures [22]. These differences could affect enzyme activity and, thus, carbon uptake by the fungus and other metabolic functions, such as conidiation [18].

For these reasons, it is necessary to associate the physico-chemical properties recorded in the three mixtures composted for 180 days with *A. Niger* activity.

The pH values (Table 2), was similar in all three substrates, and ranged from 7.6 to 7.8. Despite being slightly basic, these values would not be limiting for them to be used as a substrate in horticultural seedling production or as an organic soil amendment. Several authors have reported similar values in other plant composts [23-25]. Galbraith and Smith [16] found that the pH of the culture medium, seeded with *A. niger*, increased with time, but as pH increased, fungal biomass production reduced. This scenario can be related with the results obtained in this work because, although the differences in pH in the three substrates were not statistically significant, the smallest *A. niger* population was recorded in OMA, which also had the lowest pH.

When analyzing electrical conductivity, the highest values (Table 2) were recorded in composts OM and OA (1.9 and 2 dS m^{-1}, respectively), although they fell within normal ranges for agronomic use. There are no literature references that have associated these EC ranges with alterations in *A. Niger* development.

Table 2. Physico-chemical characterization of the onion–cattle manure-alfalfa composts

	OMA	OM	OA
pH	7.8 a	7.6 a	7.7 a
E.C.(dS m^{-1})	1.5 a	1.9 b	2 b

E.C. electrical conductivity. Different letters in the same row indicate significant differences

Regarding C and N content, it is well-known that their values depend on the baseline materials and the composting system [26]. C constitutes about 50% of the cell mass and its metabolism provides the energy required for microbial growth. In this work (Fig. 4), similar contents were detected in OM (88 $g.kg^{-1}$) and OA (87 $g.kg^{-1}$), and both were statistically higher in OMA (51 $g.kg^{-1}$).

The same trend was observed with Nt, which is essential, but in much smaller quantities so that microorganisms develop molecular structures of nucleic acids, proteins, enzymes and co-enzymes. Once again, the OM and OA composts did not differ from each other and were superior to OMA. The three composts gave values close to 10 $g.kg^{-1}$, a range that has been suggested as suitable for organic fertilizer [27-29], while the Nt content in OMA was 50% lower (4.8$g.kg^{-1}$).

The C:N, (Fig. 4), ratios presented significant differences among the three composts, and ranged between 8 and 11. These values indicate final compost maturity, which is suitable for use in agricultural production, and are consistent with those suggested by Kokkora and Hann [29].

Phosphorus and potassium are two essential nutrients for plants and, together with nitrogen, they are active ingredients of combined fertilizers used as nutritional supplements in agriculture. In the three compost mixtures analyzed herein (Table 3), the values fell within the ranges indicated by Res. 264/11 SENASA (Argentina) for composts used in agricultural production. Regarding P, the highest available level was recorded in the OM blend, where the largest *A. niger* population was also recorded. Schneider et al. [30] found that by increasing the populations of this fungus in phosphate rocks, the availability of this nutrient also increased.

Research into this topic could continue because a certain population level of *A. niger* could prove to be an appropriate tool to increase the availability and mobilization of nutrients contributed by compost.

Table 3. Characterization of the onion-cattle manure-alfalfa compost

	OMA	OM	OA
Ep (%)	0.14 a	0.18 b	0.15 a
K disp. (%)	1.05 b	0.78 a	1.12 b

Ep, Extractable phosphorus; K, available potassium; different letters in the same row indicate significant differences (p =0.05). OMA, onion-cattle manure-alfalfa; OM, onion-cattle manure; OA, onion-alfalfa

In general, these results are consistent with the research reported by Escobar-Escobar et al. (2012), who considered *Aspergillus niger* to be one of the most frequently isolated microorganisms from agricultural waste-based compost mixed with cattle manure. However, the population values recorded in the three studied materials were much lower than those published by Anastasi et al. (2005) in green waste compost (1.8 105 CFU/g of SDW). These authors recommended using the composts as organic fertilizers and indicated there was no risk of spreading pathogens to the environment and damaging crops.

4. CONCLUSIONS

Based on the evaluated physical and chemical properties, the compost prepared with onions and cattle manure waste is the most appropriate for use in horticultural production as a substrate component for seedlings and/or as an organic fertilizer. The compost that originated from degrading onion waste mixed with cattle manure waste (OM) obtained the highest *Aspergillus Niger* inoculum load values, which is probably associated with its physico-chemical composition. Finally it is concluded that the physicochemical characterization and *Aspergillus Niger* quantification results enable us to infer that these composts can be either applied to soil as an organic soil amendment or used as an organic substrate for seedlings without incurring the risk of pathogens spreading in the environment and damaging crops. This study demonstrates the importance of evaluating the fungi community in compost as an essential step to define the best application fields and to achieve the same quality by ensuring sustainable practices when applied to agricultural production.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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