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Biological control of soil-borne phytopathogenic fungi through onion waste composting: implications for circular economy perspective

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Abstract

The production of onion waste derived mainly from bulbs affected by fungal diseases, during onion classification and storage presents an important agro-environmental issue in onion production regions. Composting is an environmentally friendly strategy to recycle agricultural waste and produce organic fertilizers. Modifications of the microbial community in soil can affect the ability of pathogen propagules to survive, germinate and infect plant roots. Hence, the main objective of this work was to exploring the mechanisms involved on the presence of three soil-borne phytopathogenic fungi during the composting process of onion waste under the hypothesis if that the resulting compost effectively prevents or minimizes the dispersion of phytopathogenic fungi. To this end, three composting piles of 60 tonnes each were built by layering onion waste affected by phytopathogenic fungi and cow dung at 1:1 ratio. Temperature, moisture, pH, electrical conductivity (EC) and *Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp. growth were monitored for 100 days. During the first 28 days of composting, the presence of phytopathogenic fungi increased significantly showing thereafter a downward trend. Final estimations of fungal populations densities indicated a predominance of *A. niger* and an effective reduction in the abundance of *Fusarium* sp. This pilot-scale work demonstrates the feasibility of composting onion waste contaminated with phytopathogenic fungi and high-lights the positive environmental impact associated with this practice. Therefore, the composting of onion waste and cow dung is a feasible and sustainable procedure to recycle onion waste and to promote circular economy in onion production regions.

Keywords Onion · Phytopathogenic fungi · Biocontrol · Agricultural waste · Compost · Circular economy

Introduction

The commercialization of onion (*Allium cepa* L.) is usually done several months after harvesting. The storage and conditioning of onion produce large amounts of organic residues, due to cleaning and discard of bulbs affected by post-harvest fungi. The Valle Inferior de Río Negro region in Argentina has an onion cropping surface of 2000 ha (IDEVI 2016)

and produces about 5000 m^3 of onion waste per year. Post-harvest diseases have a negative impact on onion quality and yield a substantial amount of agricultural waste because the affected bulbs are discarded. This agricultural waste is accumulated next to agro-industrial facilities or even in agricultural soils, with consequent dispersion of phytopathogenic fungi and the ensuing environmental impact (Pellejero et al. 2011). The European Commission introduced a new concept based on the recovery of materials, i.e., the "circular economy" in response to environmental and social problems. To promote circular economy, onion waste can be recycled by composting or vermicomposting to produce environmental friendly agricultural inputs such as organic fertilizers and amendments (Pellejero et al. 2017, 2020). Since agricultural wastes are a reservoir of valuable substances (organic carbon and nutrients), composting is an efficient and agro-ecological way to recycle organic wastes through aerobic biotransformation of organic materials into a stabilized end product, i.e., compost (Román et al. 2015; Vargas-Machuca

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et al. 2015). Application of compost to agricultural soils as organic fertilizer or amendment produces noticeable agronomic and environmental benefits. These include increased transfer of soil organic carbon and reduced use of chemical fertilizers, and attenuation of negative effects of pesticides on soil microbiota and its leaching (Mondini and Sequi 2008; Carpio et al. 2020a, b).

Microorganisms are responsible for most of the physicochemical changes that occur on organic wastes during composting, govern the conversion rate of organic waste and determine the final quality of the compost (Moreno-Casco and Mormeneo-Bernal 2011). Hence, a thorough knowledge of the microbial communities involved in the composting process is paramount. The microbial consortium is typically composed of combination of mesophilic and thermophilic microorganisms such as bacteria (especially Actinobacteria) as well as diverse fungi (Haseena et al. 2016). Multiple genera of filamentous fungi with high cellulase and ligninolytic activity were identified during green compost and vermicompost analyses (Anastasi et al. 2005; Haseena et al. 2016).

During the post-harvest management of onion, substantial economic loss may occur due to several fungal diseases. *Aspergillus niger* is the most common pathogenic fungus worldwide and is responsible for the black mold on onion bulbs (Kumar et al. 2015; Lee et al. 2001; Tyson and Fullerton 2004). In recent years, other post-harvest diseases such as blue mold by *Penicillium* spp., basal rot by *Fusarium oxysporum* and blossom end rot by *Botrytis allii* are increasingly affecting onion farming (Chorolque et al. 2018). *F. oxysporum* was reported as a pathogen of onion in field and storage (Sánchez et al. 2015). This fungus survives between consecutive onion crop cycles as a soil saprophyte, in bulbs or onion wastes (Lee et al. 2001; Samuel and Ifeanyi 2015; Tyson and Fullerton 2004) which means that the inoculum formed by conidia, mycelia or resistance structures remains in the onion processing zones and increases its presence over time. The application of composting wastes to agricultural soils can control the propagation of soil-borne phytopathogenic fungi by competition between compost and soil microbiota (de Ceuster & Hoitink 1999; Pugliese et al. 2008). For example, the antagonist effect of the compost microbiota against the phytopathogenic fungi was reported in *Sclerotium rolfsii* (Hadar and Gorodecki 1991). Moreover, aerobic composting for 21 days with temperatures in excess of 70 °C

and 40 °C during 10–15 days eliminated *Armillaria mellea, Rhizoctonia solani* and *Verticillium dahliae* in green wastes (Yuen and Raabe 1984). In turn, the composting of onion wastes allowed effective control of *Sclerotium cepivorum*, which causes white rot in onion and garlic (Coventry et al. 2002; Entwistle 1990). However, there is still limited information regarding onion waste composting as a biocontrol strategy to prevent the dispersion of phytopathogenic fungi containing the discarded bulbs, as well as on the safety of the resulting compost upon application as organic fertilizer. Hence, characterization of the composting process of onion waste and assessment of the phytopathogenic load of the resulting compost are necessary to minimize the risk of dispersing phytopathogenic fungi upon its application into agricultural soil.

Along these lines, the aims of this work were (i) to assess the evolution of physicochemical parameters of the composting process and (ii) to determine the population kinetics of the main phytopathogenic fungi of onion crops during the composting process, to elucidate if the resulting compost could act as a dispersion agent. This work contributes to demonstrate a feasible strategy to recycle agricultural wastes with the further reduction of chemical inputs in agriculture and promoting circular economy and organic farming.

Materials and methods

Composting method, location and experimental design

In this study, we evaluated composting from two biowastes that result of regional agricultural activities and constitute a serious environmental problem: onion crops residues and cow dung. The composting has two purposes: To produce compost as a procedure to recycle onion waste promoting circular economy in onion production regions and to control soil-borne pathogens affecting the onion crop.

Compost can be produced from many different types of biowastes, including crop residues. Common methods of on-farm composting include static piles, windrows (elongated piles) and in-vessel (enclosed) composting (Cooperband 2002). The composting method used in the assays was *windrows piles with aeration by means of periodic turns.* This method is the most common among onion producers due to

Fig. 1 General aspect of the onion waste **a**, construction of composting piles by alternating layers of onion waste and cow dung **b** and irrigation system **c**

the large amounts of residues they generate. Furthermore, the method is inexpensive and easy to implement.

For this, the assays were performed at pilot scale at the Mandurai S.A. facilities in San Javier, Argentina (40°47' S; 63°12' W). The discard from 180 ha of onion crop, i.e., cataphylls and bulbs affected by post-harvest fungi (Fig. 1a), was used to build the composting piles. Three composting piles (30 m long × 3 m wide × 1.5 m high) were built by alternating layers of onion waste and cow dung (Fig. 1b). The final weight of each pile was approximately 60 t. The ratio of onion waste: cow dung was determined according to Rynk et al. (1992) to reach a final C/N ratio of 30. Cow dung is a suitable material to help the compost in the maturity process and was previously used to composting onion waste and other organic wastes (Pellejero 2015; Abdoli et al. 2019). The final ratio of onion waste:cow dung was 1.3:1 (w:w). C and N were determined by combustion (Bremner 1996). The moisture of the composting piles was kept constant by aspersion irrigation (Fig. 1c). The composting process took place for 100 days from March to May 2017.

Sampling and physicochemical analysis

Temperature was measured daily using a digital thermometer placed at a depth of 30 cm at different sites of the composting piles. Sampling was performed according to the recorded temperatures, indicative of the composting phases (i.e., thermophilic, mesophilic and maturation). Composite samples were taken from each pile, the days between one sampling and another vary depending on the phases of the composting process. One sampling was performed at the beginning of the process (day 0), four during the thermophilic phase (9, 20, 28 and 38 days) and six during the maturation phase (54, 65, 70, 77, 88 and 100 days). For each composting pile, three different composite samples (1 kg each) were obtained by mixing individual sub-samples from the central area and from two margins of the composting pile to a depth of 30 cm. Moisture was determined by lost weight at 105 °C whereas pH and EC were determined in 1:10 (w:v) extract using a pH meter (Hanna, HI-99141) and a conductivity meter (Hanna, HI-2316), respectively.

Extraction and count of phytopathogenic fungi during composting

Identification and count of phytopathogenic fungi were carried out using four sub-samples from composite samples obtained from each composting pile as described above. The extraction was performed by vigorously mixing 10 g of each sample in 90 mL of sterilized water, followed by serial dilution up to 10^{-6}. Each sample was extracted in quadruplicate, to obtain a total of 12 samples for each sampling time.

Aliquots (1 mL) of each dilution were inoculated per triplicate in petri dishes containing potato dextrose agar (PDA) and incubated at 24 °C for 7 days in the dark (Trombetta et al. 1998). The identification of colonies was performed according to macro- and microscopic morphological characteristics of fungi. Colony forming units (CFU) counts for each tested fungi (*Aspergillus niger*, *Penicillium* sp. and *Fusarium* sp.) were normalized against each dilution factor and the results expressed as CFU per gram of dry compost.

Statistical analysis

Moisture, pH and EC data were analyzed by one-way analysis of variance (ANOVA). Tukey post hoc test with a level of significance of 95% ($p \leq 0.05$) was used to determine differences between the three piles at both identical and successive sampling times. To determine temporal variations in phytopathogenic fungal populations during the composting process, the respective CFU count results were statistically evaluated by one-way ANOVA followed by Tukey post hoc test, with a significance threshold of $p \leq 0.05$. All the statistical analyses were performed using INFOSTAT software (FCA, UNC, Córdoba, Argentina).

Fig. 2 Temperature **a** and moisture **b** measurements during the composting of onion waste mixed with cow dung. Bars indicate standard deviation ($n = 3$)

Results and discussion

Physicochemical evaluation of the onion waste composting process

Temperature and moisture profile

Temperature is a key indicator of the various stages of the decomposition process of organic matter and determines the degree of stability of the final end product (Moreno-Casco and Mormeneo-Bernal 2011; Chin et al. 2020). Another important factor is moisture, which critically impacts microbial growth and metabolism within the composting pile. For instance, excess moisture reduces the porosity of the compost mix, generating an environment that enhances anaerobic growth (Márquez-Bueno and Díaz-Blanco 2007).

Temperature profiles recorded during the composting of onion waste and cow dung are shown in Fig. 2a. These data indicate that the mesophilic phase occurred over the first two composting days, the thermophilic phase took place between the 3rd and 45th days, and the maturation phase encompassed the remaining period up to the end of the assay (100 days). The three composting piles showed similar temperature profiles during the composting process. Compost temperature ~ 60 °C by the 3rd day of the thermophilic phase, denoting high activity of the indigenous microbial population. This is in agreement with the general increase in the microbial population that occurs in the early stages of composting, reflecting adequate nutrients availability and suitable environmental conditions (Chin et al. 2020). During the thermophilic phase, temperature remained above 50 °C for 6 days. Several studies showed that temperature increments during composting increase the rate of organic matter conversion and reduce the population of pathogenic microorganisms (Dehghani et al. 2012; Sinhg et al. 2015; Tremier et al. 2005). After 8 more days, the temperature of the composting piles remained between 30 and 40 °C for the following 31 days. The piles were flipped on day 35 of composting in order to increase the porosity and oxygen availability in the composting material. This process increased microbial activity, as reflected by a small increment in temperature. After the thermophilic phase, the temperature gradually decreased to remain stable at around 15 °C until the last day of composting.

Moisture levels ranged were between 34 and 42% at the initial time and increased up to 50% during the first 20 days (Fig. 2b). These values are in agreement with of 30–70% moisture range recommended by Martínez-Nieto et al. (2011) for the initial composting stages. In this study, moisture fluctuations were controlled by sprinkler irrigation and set gradually over time at around 45%. No significant differences in moisture content were observed for the three piles
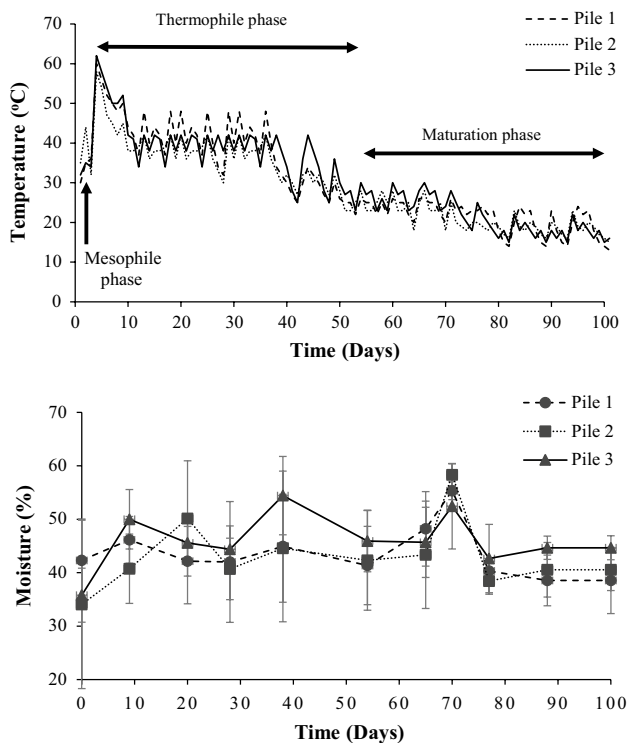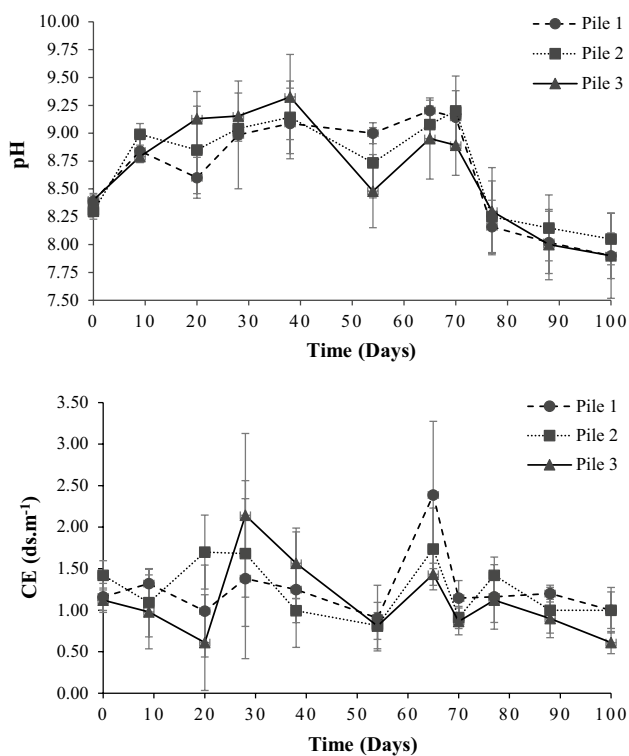
Fig. 3 pH **a** and electrical conductivity (EC) **b** measured in the three piles during the composting of onion waste mixed with cow dung. Bars indicate standard deviation ($n = 3$)

evaluated in the assay ($p \geq 0.05$), except toward the end of the composting process (88–100 days) when the moisture of the composting pile 3 was significantly higher than that of piles 1 and 2 ($p \leq 0.05$). However, this difference did not have obvious implications regarding final compost quality. Indeed, and as recommended by previous works (Guo et al. 2012; Richard et al. 2002), moisture content during the composting process ranged from of 40%–60% for the three piles. Moisture control is crucial during the composting piles to enhance microbial activity and aid in the decomposition of organic matter. Moisture values below 30% inhibit microbial activity, preventing decomposition of organic material. Conversely, moisture levels above 65% decrease porosity and prevent adequate gas exchange, reducing oxygen availability and promoting the growth of anaerobes (Miyatake and Iwabuchi 2006).

Variation of pH and electrical conductivity

pH and EC are two of the main variables affecting compost quality. pH is an important parameter to assess the microbial environment of composting and the degree of stability reached by organic wastes. EC is an indicator of soluble salt content and is determined by the composition and nature of the feedstock.

The pH variations during the composting process are shown in Fig. 3a. Initial pH values for the three piles were very similar, (8.30–8.40) denoting the homogeneity of the corresponding onion waste / cow dung mixtures. In the mesophilic and thermophilic phases, pH values increased significantly ($p \leq 0.05$) reaching maximum between days 38–77 in the three piles. This increment can be attributed to degradation of organic acids and production of ammonium derived from protein degradation. Similar values of pH were obtained during the composting of hen manure (Álvarez-Vera et al. 2019). The decomposition of organic matter is inhibited at low pH, hence pH values higher than 7.5 during the composting process are indicative of good environmental conditions (Dias et al. 2010; Pellejero et al. 2015). During the maturation phase, pH decreased due to removal of ammonium via nitrification (Cayuela et al. 2007; Villanueva 2015). Accordingly, a significant drop in pH (in excess of one pH unit) was detected during the last 30 days of the composting process. The final pH values recorded (close to 8.0), reflected in turn the maturity of the final compost (Varma and Kalamdhad 2014). Generally, the pH of compost piles increases at the beginning of the composting period and then shows a slight drop (Chin et al. 2020). Our results are in agreement with those achieved during the composting of vegetable waste mixed with cattle manure (pH 7.7–8.0) (Sudharsan Varma and Kalamdhad 2015). Of note, a final product with pH 7.8, similar to that registered in the present work, was obtained after vermicomposting of onion waste mixed with cow dung (Pellejero et al 2020).

The EC profiles for the three composting piles are shown in Fig. 3b. Although significant inter-sample differences in EC values were recorded at several time points, the three composting piles showed comparable EC profiles that tended to vary in parallel over the course of the composting process.

During the thermophilic phase (i.e., between days 3 and 45 of composting), the EC increased to reach 2.14 dS m^{-1}. EC elevations during this composting phase are due to decomposition of organic matter which determines an
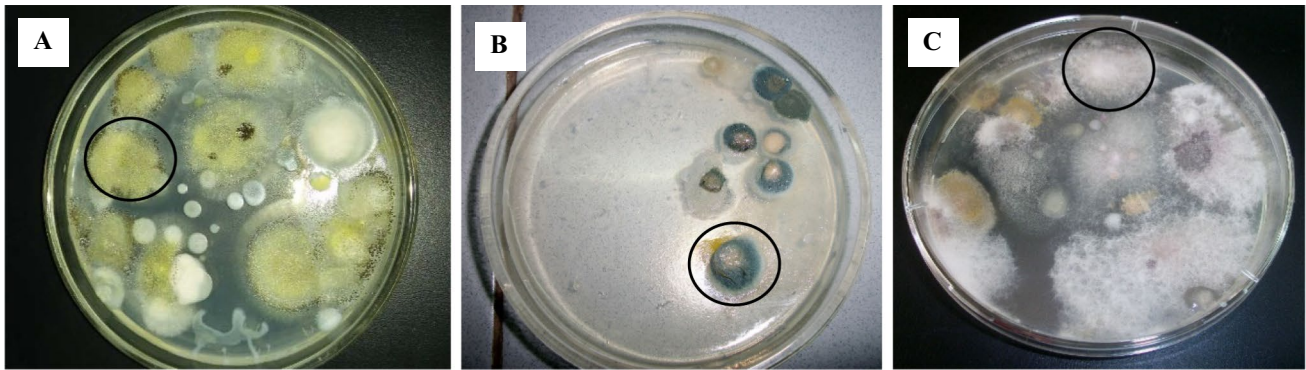
Fig. 4 Visual aspect of colonized petri dishes (ø 15 mm) used for the identification and count of phytopathogenic fungi: *Aspergillus niger* **a**, *Penicillium* sp. **b** and *Fusarium* sp. **c**. Circles indicate colonies of the corresponding fungi
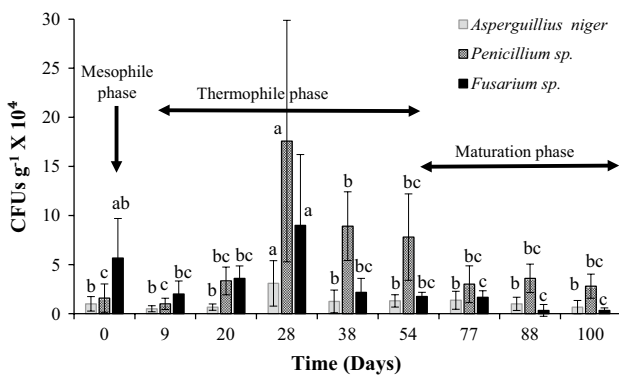

Fig. 5 Time course assessment of phytopathogenic fungi populations during the composting of onion waste and cow dung. Bars indicate standard deviation ($n = 12$). Different letters indicate significant differences among sampling days for each fungus (Tukey post hoc test, $p \leq 0.05$)

increase in soluble salt content that is not attenuated by leaching or binding to stable organic complexes (Dadi et al. 2019; Sudharsan Varma and Kalamdhad 2015). The EC of a compost mixture is primarily determined by its salts content, and by ions such as ammonium and nitrate, produced during the composting process (Sánchez-Monedero et al. 2001). At the transition between the thermophilic and the maturation phases, a second increment in EC was observed in the three piles, which likely resulted from the turning of the compost earlier on. From day 77 of composting and until the end of the process, EC decreased significantly ($p \leq 0.05$). The decrease in EC at the maturation phase could be attributed to

a reduction in soluble salts contents through leaching caused by irrigation of the piles (Alburquerque et al. 2006). The final EC of pile 3 was significantly lower than those recorded for piles 1 and 2. This could be related to the higher moisture content of pile 3 at this point, and therefore, to increased salt leaching relative to piles 1 and 2. Nevertheless, no significant issues are expected to arise from the agricultural application of any resulting composts.

Assessment of phytopathogenic fungi during onion waste composting

The presence of three soil-borne phytopathogenic fungi affecting post-harvest onions, i.e., *A. niger*, *Penicillium* sp. and *Fusarium* sp., during the composting of onion waste mixed with cow dung was analyzed by assessing CFU variation over time in water extracts from the three composting piles herein assayed. For the three fungal genera, CFU counts were performed at a 10^{-4} dilution, which provided the best visualization of individual colonies (Fig. 4). The corresponding data are shown in Fig. 5. The usefulness of this methodology is supported by studies assessing variations in microbiota composition associated with temperature changes during the different composting phases (Moreno-Casco and Mormeneo-Bernal 2011). Our analysis showed that at the beginning of the composting process, namely during the mesophilic phase, the most abundant fungus was *Fusarium* sp. followed by *Penicillium* sp. and *A. niger*. Over the first days of the thermophilic phase (sampling day 9), the piles reached their maximum temperature (58–62 °C)

(Fig. 2a). Such high temperature rage, prolonged over several days is likely responsible for the observed decrease in the phytopathogenic fungi population. Despite relatively large reductions (between 37 and 65%) in CFU counts from baseline to composting day 9, the decrease was not significant for any fungus ($p > 0.05$).

For the three fungi, CFU counts increased significantly in the thermophilic phase between days 20 and 28 with temperatures below 50 °C ($p \leq 0.05$), reaching a maximum at 28 days of composting. In turn, a significant decrease in CFU numbers was observed in all three cases at subsequent sampling times ($p \leq 0.05$). Previous research that indicated that *Aspergillus* sp. and *Penicillium* sp.*,* followed by *Trichoderma* sp.*, Mucor* sp.*, Rhizopus* sp.*, Cladosporium* sp.*, Backusella* sp.*, Ulocladium* sp.*, Acremonium* sp.*, Fusarium* sp.*, Scopulariopsis* sp. and *Geotrichum* sp. are the predominant fungal genera in this composting phase (Anastasi et al. 2005; Haseena et al. 2016; Rouhullah et al. 2012). Our results are in good agreement with the above genera distribution and reproduce the noticeable fungal population reduction that is reported to occur as composting time progresses (Moreno-Casco and Mormeneo-Bernal 2011). In our work, the maturation phase was characterized by a gradual decrease in the number of the phytopathogenic fungi which was toward the end of the process significantly ($p \leq 0.05$) lower than in the previous phases. Chin et al. (2020) reported a significant (onefold to twofold) decrease in fungal population numbers and an increment of the Actinobacteria population in the last stages of the composting process. In this work, a marked decrease in the *A. niger* and especially the *Fusarium* sp. populations was evident in the final compost product, with both genera attaining CFU values of $< 10^4$ CFU g^{-1}. Of note, these values are lower than those reported by Anastasi et al. (2005). For all, the composting process of onion waste mixed with cow dung did not increase the abundance of the three phytopathogenic fungi. Moreover, this process significantly reduced the CFU of *Fusarium* sp.

The most abundant fungus in the final compost was *Penicillium* sp. According to Gómez-Griñán (2004), the presence of the genus *Penicillium* is beneficial, since it promotes the solubilization of phosphorous from organic matter by acid- and alkaline-phosphatases, acting therefore as a biostimulant.

Implications for circular economy and organic farming

The present work deals with the principles of the circular economy because two agricultural wastes, onion waste and cow dung, are recycled and reintroduced into the productive system as a new raw material, i.e., compost. Composting is one of the best and most sustainable methods of agricultural waste recycling because it allows reincorporating nutrients and organic matter into circulation while maintaining the waste–soil–plant production continuum (Jakubus 2020). The use of compost is within the scope of a sustainable agricultural system that provides an ecologically healthy and economically viable crop production, especially when the latter derives from low-value by-products of the cultivated plants (Kadoglidou et al. 2019). From an economic perspective, it should be underlined that raw materials used for composting are easily available locally. Thus, the final product is relatively cheap and marketable by farmers, providing a cost-effective alternative to commercial mineral fertilizers (Jakubus 2020).

There are multiple current and proved experiences of successful recycling of agricultural waste through composting. For example, Kadoglidou et al. (2019) reported that the application of compost from rice by-products on rice paddy fields satisfies crop nutrient requirements, without compromising yield and quality. Meanwhile, Duque-Acevedo et al. (2020) reported that self-management of horticultural waste by composting is profitable at real scale. Therefore, converting agricultural wastes into organic fertilizer helps to break the linearity of the traditional agricultural system and to close the loop for a more sustainable crop production. Hence, agricultural waste recycling for composting represents a feasible, cost-effective strategy to minimize the use of chemical fertilizers, reduce greenhouse emissions and enhance carbon sequestration with the consequent reduction in the environmental impact (Diacono et al. 2019; Thomas et al. 2019). However, for a full and wide-scale implementation of this practice, recycling policies must be enacted and reinforced at different government levels (Duque-Acevedo et al. 2020; Jakubus 2020).

Conclusion

Our study shows the feasibility of turning discarded onion waste affected by phytopathogenic fungi into organic fertilizer suitable for agricultural practices. The physicochemical data collected during the 100 days composting of onion waste and cow dung indicate both a correct composting process and a stable compost product. Although further in-field testing is warranted, the adequate pH and moisture levels, as well as the low electrical conductivity, recorded over the process suggest that the compost produced in our work is adequate for agricultural input as organic fertilizer or amendment. Importantly, our work demonstrates that the composting of onion waste and cow dung in a ratio of approximately 1:1 does not act as multiplicator of phytopathogenic fungi. Moreover, the composting process presented herein was effective in reducing the presence of *Fusarium* sp., a phytopathogen of particular concern in onion production. Hence, the composting of onion waste and cow dung is a feasible and sustainable procedure to recycle onion waste and to promote circular economy in onion production regions. In the future is need to identify the patterns in the relationships between the microbial diversity and ecosystem services; also, quality standards are required in order to avoid phytotoxicity effects on plants.

Availability of data and material (data transparency) Under request.

Code availability (software application or custom code) Not applicable

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

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