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EVALUATION OF MICROBIOLOGICAL QUALITY OF *LACTUCA SATIVA* LEAVES CULTIVATED WITH COMPOSTS OF POULTRY MANURE IN SOUTHERN BENIN

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ABSTRACT

Raw poultry manure are widely used in market gardening in southern Benin, these manure can pose great health risk if they come from infected or sick animals. Indeed, they can contaminate vegetables on which they are spread and therefore endanger consumers' health. Hence the need to compost poultry manure before using them in market gardening. The main objective of this study is to evaluate microbiological quality of Lactuca sativa leaves (lettuce) cultivated with composts of poultry manure. To this end, compost and digestate were produced with poultry manure respectively by aerobic composting and anaerobic digestion atthe Center of Waste Valorization into Renewable Energies and Agriculture (University of Abomey-Calavi) and they were used to cultivate lettuce on the same site. Lettuce was also cultivated in the peasant environment, in two market gardening centers located in Abomey-Calavi and Fidjrossè, using compost produced in each of these two centers. On these three different market gardening sites, the crop was also made with raw manure in parallel with composts produced. On each site, samples of lettuce were collected and sent to the laboratory for the counting of total flora, Escherichia coli, coagulase-positive Staphylococcus, Clostridium perfringens and for the detection of Salmonella spp. Use of different composts made it possible to obtain lettuces of better microbiological quality overall. However, persistence of total flora and Escherichia coli or Clostridium perfringensflora was observed on some lettuce plants amended with compost or digestate, which would be related to some external factors of contamination such as quality of irrigation water, healthiness of the cultivation soil and hygiene of the crops environment in general.

KEYWORDS: poultry manure, compost, lettuce, microbiological quality.

1 INTRODUCTION

ISSN: 2582-6271

Vol.1 No.1; 2020

Poultry farming, which was mainly practiced in rural areas in line with relatively poorly-developed livestock systems (Bebay, 2006), became an urban and periurban activity thanks to demographic explosion and boom of rural exodus, just like modern agriculture.
Due to a highly polluted farming environment, poultry farms are exposed to various diseases of bacterial and viral origin (Newcastle disease, infectious bronchitis, coccidiosis, smallpox, etc.) that pose a threat to human health. Therefore it is important to apply biosecurity measures on farms to keep animals healthy, these measures are nothing more than sanitary barriers to prevent introduction and spread of diseases or pathogens in livestock (Drouin, 2000; AMCRA, 2013).

Poultry manure, widely used in market gardening in Southern Benin because of their high availability, their competitive price and high nitrogen content (Ahouangninou et al., 2013), are mostly applied directly in the fields without any prior treatment. What is worrying is that these poultry manure, often rich in pathogenic microorganisms (Puterflam et al., 2005), may reflect the poor health of the birds from which they come if biosecurity measures are not respected. Studies have shown that the use of raw poultry manure in market gardening has an impact on both microbiological and chemical quality of produced vegetables (Métras, 2003; Kouakou et al., 2005; Dougnon, 2013). According to Kondo et al. (2006), non composted or poorly composted manure used as fertilizer could contaminate produced vegetables. Also, according to CECMA (Quebec), « vegetables and fresh fruits can be vectors of pathogenic microorganisms derived from organic fertilizers » (CQIASA, 2009).Therefore, there is a public health problem related to sanitary quality of vegetables produced with poultry manure.

The concern is major when we know that market gardeners favour production of speculations used in preparation of cold meals such as lettuce (88.5%), carrot (66.2%), cabbage (48.2%) which are the most widely grown vegetables in the two experienced market garden centers in Cotonou, Houéyiho and Akogbato (Boko, 2018).We were interested to cultivation of Lactuca sativa with poultry manure which had undergone composting prior to its application on the ground.

Cultivated lettuce (Lactuca sativa) or salad is an herbaceous plant belonging to the genus Lactuca, of the family of Asteraceae, with two distinct phases: the reproductive phase and vegetative phase. The latter, forming a more or less closed apple, is the stage used for commercialization. Lactuca sativahas several varieties that appear to be derived from a wild species, Lactuca serriola L., known as wild lettuce found in a large area including Europe, North Africa and much of Asia. More than 2160 varieties of lettuce are listed in the European official catalog of species and varieties and nearly 400 in the French catalog. Fresh lettuces are usually eaten raw in salad, they are also eaten cooked, in soup, braised or mixed with other vegetables. Lettuce is rich in nitrates that convert to nitrites through some oral bacteria. Nitrites are involved in vasodilatation and fluidification of

ISSN: 2582-6271

Vol.1 No.1; 2020

blood, which improves influx of blood in certain areas of the brain. A daily dose of lettuce can potentially prevent dementia and cognitive decline by improving this cerebral blood flow (Presley, 2010).

According to TRAME (2008), composting is a controlled biological process of conversion and recovery of organic waste (by-products of biomass, organic waste of biological origin) into a stabilized, hygienic product similar to a soil rich in humic compounds. According to the same author (2006), it is a biological technique of recycling of organic matter which, at the end of its evolution, gives humus that are factors of soil stability and fertility. Then, there are two types of composting: aerobic composting and anaerobic composting. During aerobic composting, aerobic microorganisms decompose organic matter and produced carbon dioxide (CO2), ammonia, water and heat (Misra et al., 2005). Anaerobic composting or anaerobic digestion, also known as biomethanisation, is the degradation of organic matter by microbial fermentation into a combustible gas (biogas) and a solid residue (digestate) more or less depleted in organic matter (Saidi et Abada, 2007).

The objective of this study is to evaluate micobiological quality of Lactuca sativa leaves grown with the compost of poultry manure on the soil of the experimental center of Waste Valorization into Renewable Energies and Agriculture (VALDERA) of the University of Abomey-Calavion the one hand and on two market gardening sites located in Abomey-Calavi and Fidjrossè on the other hand. Specifically, it was to check both in the cultivated soil and on lettuces, presence of thermo tolerant (also known as faecal) coliforms including Escherichia coli, of coagulase-positive Staphylococcus, Clostridium perfringens, total mesophilic aerobic flora and of Salmonella spp.

MATERIAL AND METHODS

Experimentations at the VALDERACenter

Sampling and microbiological analysis of the cultivation soil

Using a sterilized spoon, five soil samples of approximately 500 g each were collected in sterile sachets randomly in the area delimited for cultivation. They were placed in an insulating box at 4°C and taken to the laboratory for the counting of total flora (ISO 4833-1: 2013), thermotolerant coliforms (NF V 08-060), coagulase-positive Staphylococcus (ISO 6888-2 : 2003), Clostridium perfringens (NF V 08-056 : 1994) and the detection of Salmonella spp. (ISO 6579 : 2002).

Production of lettuce with compost, digestate and raw manure

At first glance, compost and digestate were produced respectively by aerobic composting and anaerobic digestion at VALDERA Center with raw manure collected on a poultry farm in Southern Benin, located about 10 km from Cotonou.

ISSN: 2582-6271

Vol.1 No.1; 2020

A lettuce nursery was made and amended exclusively with the digestate. After three weeks, the nursery was transplanted to three cultivation blocks. Each block consisted of three planks of 3.6 m2, so there was a total of nine planks of lettuce (Figure 1).By the randomization method, the planks were fertilized on each block respectively with compost, digestate and raw manure from the initial farm. The planks were watered with drilling water available on the site of VALDERA Center.

Sampling and microbiological analysis of produced lettuce

At maturation, three samples of about 500 g of fresh lettuce each were taken in sterile bags respectively on the planks amended with compost, digestate and raw manure. For each sample, the leaves were taken randomly at different locations on the set of three planks that received the same fertilizer. The leaves were cut about 5 cm from the root using a pair of sterilized scissors. The three samples were immediately stored in an insulating box at 4° C and sent to the laboratory for the counting of total flora (ISO 4833-1 : 2013), of Escherichia coli (ISO 16649-2 : 2001), of coagulase-positive Staphylococcus (ISO 6888-2 : 2003), of Clostridium perfringens (NF V 08-056 : 1994)and the detection of Salmonella spp. (ISO 6579 : 2002).

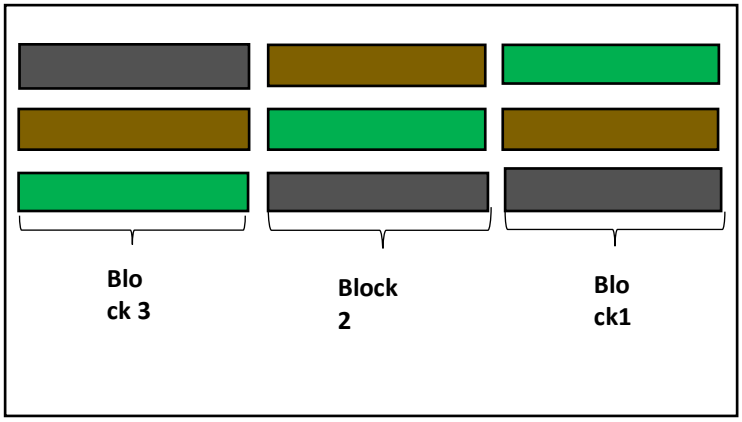


Figure 1. Scheme of the planks amendment for lettuce cultivation at VALDERA Center

Caption : Compost Digestate Rawmanure

Experimentations in the peasant environment

Realization of aerobic composting in Fidjrossè and Abomey-calavi

The aerobic composting of poultry manure was carried out at two market gardening centers respectively located in Fidjrossè and Abomey-Calavi. These two centers were chosen as pilot centers in a reasoned

ISSN: 2582-6271

Vol.1 No.1; 2020

manner, given the importance of market gardening in these two geographical areas and the activities took place with the consent of the market gardeners. The care was left to each of them to buy 10 bags of manure in poultry farms of their choice. At each site, aerobic composting was carried out as follows (Figure 2):

- a space under shade has been cleared on the site;
- two composting areas of 20 cm deep, 1 m wide and 3 m long each were dug, the pile was made in one of the holes;
- first the bottom of the hole was watered;
- a first layer of manure about 20 cm thick was spread, watered abundantly and then packed well;
- afterwards several layers of manure have been spread up to a height of 1 m approximately, taking care to water abundantly and to pack the layers one after the other;
- the pile so made was covered with palm leaves;
- a wooden stick has been inserted into the pile to control the temperature;
- the pile was returned every two weeks on the two sites;
- the turnarounds were made alternatively in the second hole and then in the first hole;
- the pile was allowed to mineralize during the rest of the time.

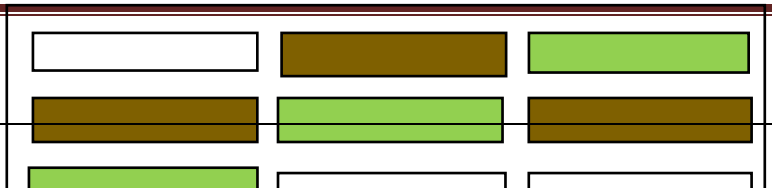
ISSN: 2582-6271

Vol.1 No.1; 2020

Figure 2. Stages of aerobic composting achievement

Production of lettuce with the composts obtained and raw manure

At the two market gardening sites, the compost obtained was used to produce lettuce. Like at VALDERA Center, production was done on three cultivation blocks, each block being composed of three planks of 3.6 m2 each. Each block contained a control plank with no fertilizer and two planks fertilized respectively with compost and raw manure (Figure 3).



ISSN: 2582-6271

Vol.1 No.1; 2020

Figure 3. Scheme of the planks amendment for lettuce cultivation at Fidjrossè and Abomey-Calavi market gardens

Sampling and microbiological analysis of lettuce produced at the two market gardening sites

On each market gardening site, three samples of about 500 g of fresh lettuce each were taken in sterile bags respectively on the planks amended with compost, raw manure and on the control plank. The samples were taken in the same way as the leaves had been taken from the site of VALDERACenter. They were immediately stored in an insulating box at 4°C and then transported to the laboratory for the counting of total flora, Escherichia coli and of coagulase-positive Staphylococcus.

RESULTS

The results of all the microbiological analyses carried out in this research were calculated according to the recommendations of the standard ISO 7218 of October 2007 and then expressed as logarithmic correspondents.

Microbiological quality of the cultivation soil and of the lettuce produced at VALDERA Center

The soil contained more than 7.5log CFU/g of total flora and less than 1log CFU/g of coagulase-positive Staphylococcus. The number of Clostridium perfringens was 3.7(± 3.4) log CFU/g, that of thermotolerant coliforms was 4.6(± 4.4) log CFU/gand Salmonella was absent.

Table I.Bacterial load (logCFU/g) on lettucesproduced atVALDERA Center according to the fertilizers used.

ISSN: 2582-6271

Vol.1 No.1; 2020

	Lettuces		
	Manure	**Compost**	**Digestate**
Total flora	7.2	7.0	6.8
Escherichia coli	2.8	1.9	2.8
coagulase+ Staphylococcus	<1	< 1	< 1
Clostridium perfringens	2.2	2.2	1.7
***Salmonella* spp.**	Absence/25g	Absence/25g	Absence/25g

The load of total flora on lettuce derived from digestate was lower than that of lettuces produced with raw manure (Table I). The number of Escherichia coli on lettuces from compost was lower than that of lettuces obtained from raw manure, with a reduction percentage of 32.1%. The load of Clostridium perfringens on lettuces obtained from digestate was 22.7% lower than that of lettuces derived from raw manure. Coagulase-positive staphylococci were almost absent on all the lettuces obtained, whether from compost, digestate or raw manure. Similarly, no salmonella was detected in the samples analysed.

Microbiological quality of lettuces produced in Fidjrossè and Abomey-Calavi

Escherichia coli was almost non-existent on all lettuce produced in Abomey-Calavi. On the site of Fidjrossè, lettuce derived from the compost bears some Escherichia coli bacteria unlike lettuce obtained in Abomey-Calavi where they are almost absent (Table II). Similarly, on both market gardens, coagulase-positive staphylococci were almost absent on all lettuce, whether with raw manure or compost as well as on control lettuce.

Table II. Bacterial load (log CFU/g) on the lettuce produced in the market gardens of Fidjrossè and Abomey-Calavi according to the fertilizers used.

	Lettuce					
	Manure		**Compost**		**Control**	
	A	**F**	**A**	**F**	**A**	**F**
Total flora	7,3	> 5,5	7,2	> 5,5	7	> 5,5
E. coli	< 1	1,7	< 1	1,6	< 1	< 1
Coagulase+ Staphylococcus	< 1	< 1	< 1	< 1	< 1	< 1
A : Abomey-Calavi ; F : Fidjrossè						

DISCUSSION

Microbiological quality of the cultivation soil and of lettuces produced at VALDERA Center

ISSN: 2582-6271

Vol.1 No.1; 2020

The results of microbiological analysis of the cultivation soil of VALDERA Center confirm that the soil is a living world. The search for microbiological specificities would have made it possible to specify the diversity of this telluric flora (anaerobic flora, molds, etc.). This soil contained almost no coagulase-positive staphylococci unlike the results of Dougnon et al. (2014) who detected at least 2 log CFU/g of these bacteria in the cultivation soils of an experimental field in Abomey-Calavi.

Hygienisation of the poultry manure had a positive impact on lettuces quality because lettuce that was grown with compost and digestate was less contaminated by total flora than lettuce grown with raw manure.

Since lettuces are consumed cold and Escherichia coli is much involved in food poisoning on one hand (Kondo et al., 2006) and in the occurrence of gastroenteritis related to the production of verocytoxin (Houngbegnon et al., 2016) on the other hand, the presence of this bacterium on lettuce obtained either with compost or with digestate is worrying despite the relatively low number detected on these lettuces. After consuming such lettuces, children under 5 years old for example, whose immune system is not yet sufficiently developed, can develop anuremic haemolytic syndrome (UHS) induced by shigatoxin-producing Escherichia coli strains (Niaudet, 2008, Bryan et McAdam, 2015). Contrary to this result, a study by Samake et al. (2011) in Bamako showed that the level of lettuce contamination by faecal coliforms, in urban and peri-urban areas, exceeds by a long way acceptable levels of contamination. The number of Escherichia coli on lettuce grown with compost or digestate should be less than or equal to 1 log CFU/g or not more than 2 log CFU/g as recommended by standards (Guiraud 2003, CQIASA 2009). Contrary to the absence of salmonella in all lettuce samples analysed in our study, a high prevalence of salmonella, up to 56%, was detected on some lettuces by Abdelkader et al. (2017) in some urban and peri-urban areas of Niger.

At present, there are few data on microbiological criteria for raw vegetable products (Guiraud, 2003) such as lettuces. According to CECMA (Quebec), "unprocessed raw vegetables and fruits are not likely to allow the growth of pathogenic microorganisms when they maintain their integrity" (CQIASA, 2009). The available data mainly relate to processed raw fruits and vegetables (4th range products) and fresh fruit salads (Guiraud, 2003).

Microbiological quality of lettuces produced in Fidjrossè and Abomey-Calavi

The analysis results of lettuces produced in Fidjrossè and Abomey-Calavi showed that the compost produced at both sites was of good quality and allowed to obtain lettuces of satisfying hygienic quality. The loads of total flora detected on all lettuces obtained at both sites are justified because these lettuces were produced on a soil containing between 5 and more than 7 log CFU/g of total flora. The residual

ISSN: 2582-6271

Vol.1 No.1; 2020

load of Escherichia coli detected on lettuces derived from the compost in Fidjrossè could be attributed to the possible presence of the bacterium in irrigation water or the cultivation soil.

Although the quality of irrigation water was not determined in our study, their involvement in the microbiological contamination of market gardening products is likely. In Southern Benin, the shallows water as well as drilling waters used to irrigate market gardening crops are often highly polluted. For example, in the area of Fidjrossè, shallow and drilling waters contain respectively up to 5.2 and 4 log CFU/100 ml of thermotolerant coliforms(Dougnon, 2013). Also, Moustier (2004) reported that in Yaoundé, waters used for irrigating crops in the shallow of Nkolbisson contain many microorganisms indicating faecal contamination. In Dakar, on one hand, large concentrations of bacteria were found in irrigation waters of many plots (Niang, 1992); on the other hand, some amoeba cysts, larvae of eels and eggs of parasites were detected on raw consumable vegetables watered with wastewater (Niang, 1999). According to Ndiaye (2009), 35% of irrigation waters used in urban agriculture in Dakar were contaminated with Salmonella spp and the load of Escherichia coli on lettuces from the fields and markets of the city exceeded ICMSF (International Commission on Microbiological Specification for Food) standards.

CONCLUSION

The use of poultry manure composts has resulted in lettuces of better microbiological quality. However, persistence of total flora and non-elimination of almost all the loads of Escherichia coli or Clostridium perfringens observed on some lettuce plants grown with compost or digestate suggest a probable contamination by irrigation waters or the cultivation soils. To avoid any risk of food poisoning, it is strongly recommended to properly disinfect lettuces before eating them. To this end, in order to evaluate the effectiveness of disinfectants, in this case bleach, on bacterial flora of lettuces, we carried out a complementary study in laboratory whose results will be published later. In perspective, we plan to study the effect of the use of poultry manure composts on the microbiological quality of other market garden crops such as cabbages and carrots.

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