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Microbiological Quality of *Lactuca Sativa* and *Eruca Sativa* Produced in the Market Gardening Perimeters of the City of Ndjamena (CHAD)

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Abstract

Diseases associated with the consumption of contaminated fruits and vegetables are common in many regions of developing countries. In Ndjamena, market gardening concerns several types of products which contribute to the balance of the diet either in the complementation or the seasoning of the sauces or in the constitution of starters. Similarly, several vegetable species have been identified. However, lettuce (*Lactuca sativa*) and arugula (Eruca *sativa*) have been identified as the main vegetables grown by market gardeners for household consumption. The present study was carried out to evaluate the microbiological quality of *Lactuca sativa* L (lettuce) and Eruca *sativa* Mill (rocket) produced in the urban market gardening farms of Ndjamena (Chad). To achieve the set objective, we undertook an experimental descriptive cross-sectional study. Therefore, 50 lettuce samples and 50 arugula samples were analyzed. The presence of *Escherichia coli, Staphylococcus aureus, Aeromonas spp* and *Salmonella spp* was revealed. Microbiological analyzes showed a high load of pathogenic germs in the lettuce and arugula produced in the various sites of the city of Ndjamena. In addition, 19.44% of the samples of each species were unsatisfactory. In the two samples (the latitude and the rocket) Escherichia coli. were positive with 13.88% and 8.33% respectively. Salmonella was positively found in lettuce and arugula also with 8% and 12% respectively. Then about *Staphylococcus aureus* and 5.55% and 2.77% of samples are unsatisfactory in re *Aeromonas spp*. However, contamination varies between sites and depends on the environmental condition. The quality of these fresh vegetables produced is a great scourge for public health. Therefore, the risk is intensified among consumers who do not respect the hygienic conditions.

Keywords: *Lactuca sativa*; Eruca *sativa*; microbiological quality; N'Djamena; Chad

Abbreviations: EMB: Eosine Methylene Blue; XLD: Xylose Lysine Desoxycholate

Introduction

The importance of fresh vegetables for a healthy and balanced diet is widely recognized. They provide fiber, vitamins, minerals, whose recommended nutritional intakes have been established [1]. There is considerable evidence of health and nutritional benefits associated with their regular consumption [2,3]. Despite the benefits of eating vegetables, many Studies have shown that these vegetable products, consumed fresh, constitute a favorable environment for the growth of pathogenic germs. They are recognized as potential sources of foodborne illness [4,5]. Vegetables can be contaminated with pathogenic microorganisms during ripening, harvesting or sale [6]. Consumers are at risk of food poisoning. Therefore, the availability of healthy and nutritious food is one of the fundamental human rights and an essential factor for an adequate state of health [7]. Illnesses associated with the consumption of contaminated fruits and vegetables are common in many parts of developing countries, but they are

underestimated due to the lack of reliable data from surveys and surveillance [8]. In Ndjamena, market gardening concerns several types of products that contribute to the balance of the diet either in the addition or seasoning of sauces or in the constitution of starters. Several vegetable species have been identified. However, lettuce (*Lactuca sativa*) and arugula (Eruca *sativa*) have been identified as the main vegetables grown by market gardeners [9]. Faced with production constraints in urban areas and to meet ever-increasing demand, market gardeners are adopting a strategy to improve their productivity without considering several considerations to minimize health impacts. The proximity of roads, the use of wastewater and untreated organic fertilizers raise the suspicion that the contamination of vegetable crops may be critical. It is the source of the presence of pathogenic microorganisms. For this reason, the characterization of the microbiological quality of market garden products is necessary to ensure their safety. This study was devoted to the evaluation of the microbiological quality of lettuce and arugula, the main vegetables produced on the market gardening sites of N'Djamena. The microbiological quality of these fresh vegetables was assessed by determining the microbial loads and the prevalence of four presumed pathogenic microorganisms: *Escherichia coli, Staphylococcus aureus, Salmonella spp* and *Aeromonas spp*. Hence, the objective of this study is to contribute to the improvement of the quality of fresh vegetables from urban market gardening farms in N'Djamena.

Material and methods

The samples consisted of 50 samples of *Lactuca sativa* L. (lettuce) and 50 samples of Eruca *sativa* Mill. (rocket) from urban vegetable farms in N'Djamena. These samples were taken under sterile conditions at maturity at five urban market gardening sites (site I, site II, site III, site IV, site V) to consider the heterogeneity of the contamination of fresh vegetables. The choice of market gardening sites was made according to the nature of the water source for irrigation and the size of the sites. The microbiological analyzes were carried out at the Food Science and Nutrition Research Unit of the Research, Diagnostics and Scientific Expertise Laboratory of the University of N'Djamena. The methods for researching and counting germs have been the standardized AFNOR reference methods [10,11]. The presence of *Escherichia coli, Staphylococcus aureus, Aeromonas spp* and *Salmonella spp* has been studied.

For all analyses, 25 g of each sample was taken, ground and homogenized in 225 ml of buffered peptone water (EPT) in a stomacher. for one to three minutes then left to stand for thirty minutes (30 min) to allow revivification. Cascading decimal dilution series from 10 -1 to 10 -6 were made under aseptic conditions from 1 ml of each stock suspension and used for the enumeration of microorganisms. Eosine Methylene Blue (EMB) medium was used for E. coli, Chapman medium for staphylococci, Xylose Lysine Desoxycholate (XLD) medium for *Aeromonas spp* and finally for Salmonella, XLD and Hektoen media were used. The interpretation of the results was made considering the criteria defined by the Canadian regulations as indicated in the document. The criteria are therefore determined according to the risk assessment, depending on the situation [12]. To assess the microbiological quality or compliance of the analyzed vegetable samples, a two-class plan was used for Salmonella spp. and a three-class plan was used for E. coli, Staphylococcus aureus, and *Aeromonas spp* (Table1). Samples of vegetables of unsatisfactory or corrupt microbiological quality were considered as samples of non-compliant microbiological quality.

Table 1: Microbiological criteria used.

Threshold	Average load in CFU/g			
	E.coli	Aeromonas spp.	Salmonella spp.	St. aureus
m	10^2	10^2	Absence	10^2
M	10^3	10^3		10^3

m: fixed microbiological criterion
M: limit of acceptability beyond which the results are no longer considered satisfactory, of good microbiological quality.

Results

Average loads of pathogenic germs in lettuce and arugula

The results of the strain count (Table 2) showed an average E. coli load of 6.81×10^2 CFU/g and 5.27×10^2 CFU/g respectively in lettuce and arugula. For *Aeromonas spp*, the mean values obtained are of the order of 1.47×10^2 CFU/g in lettuce and 1.21×10^2 in arugula considering the tolerance threshold set at 10^3 CFU/g. As for Salmonella, the average values of the loads obtained are 0.25 CFU/g in lettuce and 0.16 CFU/g in arugula. Staphylococci (Staphylococcus aureus) are present with an average load of 2.62×10^2 CFU/g in lettuce and 1.69×10^2 CFU/g in arugula.

Contamination of lettuce and arugula by E. coli

Tables 3 and 4 give the levels of contamination of lettuce and arugula by E. coli. Overall, respectively for lettuce and arugula 55.55 and 50% of the samples are satisfactory (flora < 10^2) 25 and 30.55 % are acceptable (10^2 < flora < 10^3) while 19.44 % of each sample are unsatisfactory (flora > 10^3). For lettuce, Table 3 shows that site V is the one that gives the most satisfactory

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samples (85.71%). On site I, on the other hand, we find the most unsatisfactory samples (57.14%). Regarding Arugula, Table 4 shows that site III is the one with the most satisfactory samples (85.71%), while site I has the most unsatisfactory samples (50%).

Table 2: Average load of *E. coli, Aeromonas spp. Salmonella spp.* And *Staphylococcus aureus* in lettuce and arugula (in CFU/g).

Germs\Vegetables	E.coli	Aeromonas spp.	Salmonella spp.	St. aureus
Lettuce	6.81 10^2	1.47 10^2	0.25	2.62 10^2
Rocket	5.27 10^2	1.21 10^2	0.16	1.69 10^2

Table 3: Enumeration of E. coli in lettuce at production sites (in %).

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	14.28	42.85	62.5	71.42	85.71	55.55
10^2 < flora < 10^3	28.57	28.57	25	28.57	14.28	25
Flora > 10^3	57.14	28.57	12.5	0	0	19.44

Table 4: Enumeration of E. coli in arugula at production sites (in %)

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	12.5	71.42	85.71	14.28	71.42	50
10^2 < flora < 10^3	37.5	28.57	14.28	42.85	28.57	30.55
Flora > 10^3	50	0	0	42.85	0	19.44

Contamination of lettuce and arugula by Staphylococcus aureus

The count of presumed pathogenic staphylococci in lettuce and arugula is shown in Tables 5 and 6 and is presented as follows:

- 75.00 and 72.22% of the samples are satisfactory (flora < 10^2 CFU/g).
- 11.11 and 19.44% of the samples are acceptable (10^2 < flora < 10^3 CFU/g).
- 13.88 and 8.33% of the samples are unsatisfactory (flora > 10^3 CFU/g).

Table 5: Enumeration of Staphylococcus aureus in lettuce at production sites (in %)

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	14.28	57.14	100	100	100	75
10^2 < flora < 10^3	42.85	14.28	0	0	0	13.3
Flora > 10^3	42.85	28.57	0	0	0	13.88

Table 6: Enumeration of Staphylococcus aureus in arugula at production sites (in %)

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	37.5	100	100	28.57	100	72.22
10^2 < flora < 10^3	50	0	0	42.85	0	19.44
Flora > 10^3	12.5	0	0	28.57	0	8.33

In lettuce, the most unsatisfactory samples are found, especially at site I with 42.85% of the samples analyzed. On the other hand, this presumed pathogenic germ is not found in lettuce and arugula sampled from sites III and V. It is also absent in lettuce from site IV and arugula from site II. In short, for each species, this presumed pathogenic germ is present in two (2) sites.

Contamination of lettuce and arugula by Aeromonas spp

Aeromonas search results spp in lettuce and arugula are given in Tables 7 and 8. In general, respectively:

- 75 and 83.33% of the samples are satisfactory (flora < 10^2 CFU/g).

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- 5.55 and 2.77 % of the samples are unsatisfactory (flora > 10^3 CFU/g).
- 19.44 and 13.88% of the samples are of acceptable quality (10^2 < flora < 10^3 CFU/g).

100% of site V samples are satisfactory. Regarding rocket, *Aeromonas spp* is present in two (2) sites.

Contamination of lettuce and arugula with salmonella

Tables 9 and 10 give the results of the enumeration of Salmonella in lettuce and arugula at the level of the production sites. In the case of lettuce, 96% of the samples are satisfactory (absence of salmonella in 25g), nearly 4% of unsatisfactory samples (presence in 25g). For arugula, more than 95% of the samples are satisfactory (absence of salmonella in 25g), for almost five percent (5%) of samples unsatisfactory (presence in 25g). All samples from sites III, IV and V are satisfactory, while at site III 12.5% of the samples are unsatisfactory. 94.44% of the arugula samples are satisfactory (total absence of salmonella in 25g).

Table 7: Enumeration of Aeromonas spp in lettuce at production sites (in %)

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	57.14	71.42	62.5	85.71	100	75
10^2 < flora < 10^3	28.57	28.57	37.5	0	0	19.44
Flora > 10^3	14.28	0	0	14.28	0	5.55

Table 8: Enumeration of Aeromonas spp in arugula at production sites (in %)

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Flora < 10^2	62.5	100	100	57.14	100	83.33
10^2 < flora < 10^3	37.5	0	0	28.57	0	13.88
Flora > 10^3	0	0	0	14.28	0	2.77

Table 9: Detection (in %) of salmonella in lettuce at production sites

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Absence in 25 g (satisfactory)	70	90	100	100	100	92
Presence in 25 g (unsatisfactory)	30	10	0	0	0	8

Table 10: Detection (in %) of salmonella in arugula at production sites

Germs in CFU/g	Website (in %)					
	Location I	Site II	Site III	Site IV	Site V	TOTAL
Absence in 25 g (satisfactory)	60	80	100	100	100	88
Presence in 25 g (unsatisfactory)	40	20	0	0	0	12

Discussion

Escherichia coli was detected in the two (2) types of vegetables. The presence of Escherichia coli attests to contamination of fecal origin, probably human, and therefore of the market gardener or passers-by, especially since the sites are not protected. A high load of Escherichia coli promotes spoilage of the product and constitutes a risk of the presence of pathogenic germs [13]. The concentration of Escherichia coli measured on the surface of lettuce foliage immediately after contaminating irrigation is directly proportional to that of contaminated water according to the study carried out from 2007 to 2010 on lettuce by Glémot [14]. Like E. coli, *Staphylococcus aureus* is also present in all species with a dissatisfaction rate of 13.88 for lettuce and 8.33% for arugula. Talouizte et al. [15] observed the total absence of S taphylococcus aureus on lettuces irrigated with urban wastewater from the city of Fez in Morocco. The presence of *Staphylococcus aureus* most often results from the action of factors such as wind, dust and from a lack of hygiene on the part of the producers. Salmonella is present at two sites. In Niger, the prevalence of Salmonella found in lettuce are respectively in decreasing order 56% in Niamey, 33% in Gaya, 27% in Zinder, 13% in Maradi and Agadez, 10% in Tillabéri and finally 0% in Tahoua [16]. Other studies on lettuce found prevalences of 50% in Burkina Faso [17], 22% in Sokoto, Nigeria [18] and 16% in Maiduguri, Nigeria [19], respectively. The study conducted by Samake et al., [20] in the urban and peri-urban area of Bamako (Mali) detected Salmonella on all plants from all sites studied with a level of 47±4.3 to 4±2.8 depending on the

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location. The identification of Salmonella spp confirms the direct contamination of these leafy vegetables by irrigation wastewater. This detection of Salmonella spp is a serious health concern. The results obtained in this analysis show that the non-conformities noted during the study are due to the producers following a lack of qualification, competence, training and / or awareness of good hygiene practices and good agricultural practices. Labor is the "weakest link" and the most important [6, 21,22]. It is the major source of germs. It conditions the other "M's". Clean and healthy staff, trained in hygiene and good agricultural practices, are needed. The analysis of the results confirms the need for good hygiene practices and good production techniques in market gardening in urban areas. An ill-adapted hygiene policy will result in an increase in biological contamination with the possibility of the development of pathogenic microorganisms with a risk of food poisoning [23, 24,25].

Conclusion

Microbiological analyzes have shown a high load of pathogenic germs in lettuce and arugula produced in the city of N'Djamena. The sanitary quality of these fresh vegetables produced is therefore poor and presents a danger to public health. Consequently, the health risk to the consumer is intensified when consuming fresh vegetables that have not been subjected to adequate washing. The high-water content of fresh vegetables, the absence of lethal processes such as cooking to eliminate pathogenic microorganisms, do not guarantee the sanitary quality of the lettuce and arugula produced and can thus increase the risk of poisoning. These results confirm the need for good hygiene and good production techniques in urban market gardening in N'Djamena. Also, the control of microbiological hazards is considered a major component of food safety. It implies the need to implement a HACCP approach.

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