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Improvement of Vegetation Potato "Nicola" by Slaughterhouses Wastes, Treated By Biological Way

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Abstract
Improving and processing of slaughterhouse waste of red meat in Morocco are possible by highlighting biotechnology techniques. These biological techniques have the advantage of being easy and competitive with other methods used in the field of waste treatment. Thus, we were able to isolate and characterize two strains of lactic bacteria (HBL5, HBL10) and yeast strains (HL2, HL15) with a significant fermentative power. Stability of the product is obtained from the twelfth day of fermentation.
In addition, bacterial strains have a strong bactericidal effect vis-à-vis pathogens, allowing a significant improvement of the hygienic and organoleptic quality of the finished product, and the pH profile shows a remarkable evolution during fermentation to reach a stable value of 3.93. The acidity increased from 0.22% to 1.25% between the beginning and the end of fermentation.The fertilizer trials conducted by the bio-fertilizer showed a significant improvement of potato culture "Nicola" with a maximum value of 17.8 t / ha, obtained in the case of test II (15T/ha) relative to the chemical treatment and control groups.
Keywords: Slaughterhouse waste - Fermentation - Bio-fertilizer – Valorization - Potato.

1.Introduction
The recycling of wastes of animal or vegetable origin requires efficient methods to avoid hygienic and nutritional problems and respect better the environment.
Biotransformation of slaughterhouse waste is currently the safest and most economical way to stabilize the finished product for various applications.
The chemical and microbiological composition of Slaughterhouse waste of red meat raises a particular importance from researchers in the field of recycling of by-products and organic waste. In fact, these wastes can not be used directly or in a raw state because of their dangerous microflora as well as for alteration and hygien.
Biological treatment of wastes based on the ability of microorganisms to transform some compounds considered as substrates upgraded products. After the success of these types of techniques of biological transformations through anaerobic fermentation, our study is based on the use of bacterial microorganisms capable of transforming slaughterhouse waste as a stable finished product which will be designed for soil fertilization.

2. Materials and methods
2.1 Isolation and characterization of strains of lactic bacteria
The isolation and purification of lactic bacteria strains from different biotopes were performed on solid MRS (Man, Rogosa and Sharpe). Cultures of lactic bacteria were incubated at 30 ° C for 24 hours. The purification is performed after four successive subcultures of spreading in MRS agar medium.
2.2 Preparation of the raw material
Slaughterhouse wastes of red meat were collected at a rate of approximately 200kg / testing. After draining, 150kg of waste are treated in a mixture waste / molasses (20%) filled in closed barrels. Each barrel was inoculated by the most efficient strains in order to leave a headspace to facilitate the agitation of the barrels contents and prevent prospective overflow due to the rise of the product due to the exclusive production of gas during the fermentation.
Each barrel was inoculated with a mixed culture of lactic bacteria.
2.3 Choice of inoculum
Strains of lactic acid bacteria with a strong antibacterial and acidifying power are tested by combination (mixed inoculum with two saccharolytic yeast) to determine the best suited strain to test of waste fermentation and can lead the transformation of these materials to term. The tests are:
Control: natural fermentation of waste.
Tests: fermentation of waste by mixed culture (HBL5-HL2, HL15-HBL5) and (HL2-HBL10, HBL10-Hl15).
2.4 Fermentation in closed barrels
The fermentation of mixture of molasses - waste was carried out in closed barrels. Each barrel was filled to 2/3 by150 kg of mixture and lactic inoculum the most acidifying in order to leave a headspace to facilitate stirring the contents of the barrels and prevent potential overflow due to the rise of the product after the exclusive

production of gas during the fermentation.

2.5 Fermentation Follow-up

The monitoring of the fermentation during the biotransformation process of slaughterhouse wastes mixed with suitable proportions of molasses as a carbon source was performed by using the measurement of pH and acidity.

3. Characterization of bio-fertilizer

3.1 Physico-chemical characterization of bio-fertilizer

- The pH is measured by using a pH meter kind Orion Research. Its calibration at pH 4 and 7 is performed before any taken of measurement. Values are taken directly from the display cell.
- Acidity is measured by titration of 10ml of filtrate per an alkaline solution (NaOH) N /9 in the presence of Phenolphthalein as a colored indicator.
- The organic material is determined by the method of Walkly and Black. (Walkly and Black, 1934).
- Total nitrogen: essentially, the NH^{4+} and NO^{3-} are extracted by a solution of KCl. The NH^{4+} is assayed by steam stripping.
- Total phosphorus is determined by extraction of phosphorus by shiny concentrated nitric acid, the extracted phosphorus is assayed by calcimetry.
- Potassium is extracted in the same manner as total phosphorus and the dosage is carried out by flam photometry.
- Organic carbon is assayed by the method at potassium whose excess is assayed by the salt of Mohr (FeSO4).
- The determination of the non volatile dry matter content is determined by stoving at 105 ° C of a mass exactly weighing of the fresh sample.

3.2 Microbiological characterization of bio-fertilizers

Microbiological analysis is made to optimize the efficiency of applied biological processes. To do this we counted all studied floras.

- The total aerobic mesophilic flora (FMAT): This flora is a good indicator of the overall contamination of fermented waste. It is enumerated on PCA agar incubated for 24 hours at 30 °C.
- Coliforms: on lactose agar désoxycolate (DCL) incubated for 24 h at 30 ° C for total coliforms and 44 ° C for fecal coliforms .
- Fecal streptococci: counting on sodium azide incubated at 37 ° C for 48 h.
- Staphylococci: are enumerated on Baird Parker agar supplemented with egg yolk and potassium tellurite and incubated at 37 ° C for 48 h.
- The yeasts are counted on Sabouraud medium at 4% glucose incubated for 5 days at 22 ° C.
- Lactic bacteria: they are counted on agar of MRS medium and incubated for 48h at 30° C.
- Salmonella: pre-enrichment in selenite-cystine medium, followed by enrichment on tetrathionate broths, incubated at 37 ° C for 24h. The count was performed simultaneously on SS mediums incubated at 30 ° for 24.

3.3 Agronomic application of bio-fertilizer

The interest of fertilizer rich in organic matter in field crops is the role to be played in the status of cultivated soils and maintaining their long-term fertility. After choosing potato variety "Nicola", soil samples were taken in good condition to provide the producer with a report on the nutrient balance of the soil.

- The soil analysis will indicate the pH, the content in organic matter, in Nitrogen in Potassium and Phosphorus.
- On an area of one hectare, we used two doses of bio-fertilizers, Test I (10t/ha) and Test II (15T/ha). This amendment is made by comparison with test manure, test of chemical fertilizer (NPK) and a control.

Table 1: Plots amendments for an area of one hectare

Plot	**First day**	**60 days**
Control	-	-
Chimical fertilizer NPK 150 kg N/ha 150 kg P_2O_5/ha 175 kg K_2O/ha	3.125Q/ha	1,567Q/ha
Manure (25T/ha)	18,75 T/ha	6,25 T/ha
Test I (10T/ha)	6,5 T/ha	3,5 T/ha
Test II (15T/ha)	9,75 T/ha	5,25 T/ha

3.4 Plant material

Culture of potato is very easy and requires no technical knowledge. For this we have chosen a variety scoop

"Nicola". Indeed, during planting, the plants were deposited carefully; germs up, at the bottom of a furrow of 8 to 10 cm deep, and keep a space 30 cm between plants on furrows distant 50cm.

3.5 Tillage

Regarding the worked layer, we note that in recent years, tillage have evolved in order to get the possible thickest loose layer.

One month before planting, on a surface of one hectare divided into five plots, we relied on manual labor of clay soil and removing weeds.

3.6 Irrigation

The choice of the start date of irrigation depends on the state of water reserves in the soil and vegetative state of potato before flowering. Water needs of the potato evaluate between 400 and 600 mm depending on climatic conditions, soil type, and length of vegetation cycle. During germination, the necessary amount of water is low, and the mother tuber must be surrounded by moist soil.

3.7 Monitoring parameters

For each treatment, the experiment has several practical approaches for the study of the aerial parts of potato cultivation. On the one hand, non-destructive monitoring which allows to study the kinetics of size and foliar surface, and secondly, calculation of crop yield and the effect of the amendments applied to the soil at the end of the vegetative cycle.

3.8 Effects of studied amendments products on the soil

After harvest and yield calculation of both applied cultures and comparison of the fertilizer value of bio-fertilizer obtained relative to other inputs, we studied the effect of the products applied to soil, namely organic matter, total nitrogen, potassium and assimilated phosphorus.

4. Results and discussion

4.1 Isolation and characterization of strains of lactic bacteria

Strains of lactic bacteria were isolated from different biotopes namely milk, press juice and juice mixed with sugar cane. Only Gram-positive and catalase negative bacteria were selected and streaked on solid MRS medium (Man, Rogosa, Sharpe). The purified strains were tested for the production of antibacterial substances other than organic acids according to the method of the wells.The results show that among twenty strains of lactic acid bacteria, only two strains (HBL5, HBL10) that have performance characteristics. The results of the API 50C showed that the strains HBL10 and HBL5 are respectively Streptococcus thermophilus, Enterococcus devriesei, both lactic strains are selected for testing of biotransformation of slaughterhouses wastes in mixed culture with two strains of yeast.

4.2 Demonstration of the bactericidal effect of lactic acid bacteria strains

The bactericidal character study and evaluation of the antibacterial activity of strains which have a major acidifying power shows that strains HBL5, HBL10 have a strong bactericidal effect vis-à-vis the pathogens and the inhibition zone is characterized by the lack of development of any bacteria and having a very clear aspect. Strains HBL5, HBL10 have a significant inhibitory effect on E. coli strains, Staphylococcus aureus and Streptococcus (3).

This effect is evidenced by the presence of the RF of inhibition developed around the Petri dish wells (Figure 1), and that the inhibition zone is about 30 mm for the strain HBL5, and 28 mm for the strain HBL10 especially vis-à-vis the gram + pathogens (16).

In addition, the active substance was therefore regarded as a bacteriocin characterized by a large range of antibacterial activity against pathogenic bacteria particularly vis-à-vis Staphylococcus aureus (12).

4.3 Control of fermentation

- PH control

Monitoring of the fermentation during the biotransformation process of slaughterhouses wastes of red meat with molasses was made by measuring the pH and acidity.

The results of the evolution in pH and acidity are shown in Figures (2, 3).

These results show that natural fermentation took place in the mixture of waste-molasses inoculated with mixed cultures (HBL5, HBL10) (HL2, HL15) and the pH drop was clear from the third day of incubation at ambient temperatures to a value of 4.0 to 3.93 on the twelfth day of fermentation (Fig. 2) (7)

The decrease in pH of the fermentation product highlights good acidification through the process of fermentation inoculated wastes. In addition, the fermentation in the presence of powerful lactic acid bacteria and yeasts saccharolytic is usually accompanied by the production of bacteriocins that may be useful for the preservation of fermented products (11).

- The acidity

The study of the acidity profile showed a gradual evolution to reach a value of 1.25% after 10 days of fermentation; indeed the metabolites resulting from biological fermentation have masked the smell and give the

finished product an acid and fresh smell.
The fermentation assays with lactic acid bacteria have been made in the conditions of ambient temperature and pH of 6.35. After the addition of the inoculum of the mixed culture, the acidity is decreased gradually to a value of the order of 0.22 to 0.75% after 10 days of fermentation. The obtained acidification rates are somewhat normal, and remain under the threshold of a strong acidifying potential (9).
On the other hand, fermentation assays of wastes in mixed culture in the presence of saccharolytic and fermentation yeast, the profile of acidity shows a remarkable evolution for testing the mixed culture including testing inoculated mixed culture (HBL5-HL2), to reach a stable value of 1.02 to 1.25% after one week of fermentation. This increase in acidification rates can be explained by the timing of acidifying potential in lactic acid bacteria and yeasts in a synergistic effect (8).
Biological treatment of waste based on the ability of microorganisms to transform some compounds considered as substrates to upgraded products. After the success of these types of techniques of biological transformations by means of fermentation.
However, the fermentation trials are used with the right combination of lactic acid bacteria are characterized by a better development of the organoleptic characteristics of biotransformed product, this may suggest the important role of lactic acid bacteria combined with yeast to yield better results vis-à-vis stability and change of hygienic quality of the finished product.

4.4. Chemical and microbiological composition of finished product

The results obtained show that the rate of organic matter and total nitrogen after stabilization of finished product are significantly higher. The values obtained are respectively 40.17% and 4.23% for the finished product. This may be due to the difference in the initial raw material and the phenomenon of evaporation of volatile compounds during the incubation of mixture and the effectiveness of the inoculum used.
Phosphorus and potassium values were remarkable, for phosphorus is 5.32% and the potassium content is 38.03%, this may be due to the amount of molasses used as a carbon source during fermentation mixture preparation.
The study of the profile of the microbial flora during fermentation of slaughterhouses wastes of red meat showed a significant reduction of MTAF and fecal streptococci, with a total elimination of Fecal Coliforms and Staphylococci at the end of fermentation and after stabilization of pH of the finished product. The inhibition or destruction of flora having hygienic interest as is the case of spoilage flora are the consequences of the production of fermentation inhibitors and by lowering the pH to a level where most microorganisms are inhibited.

5. Agricultural application of bio-fertilizer

5.1 Soil analysis

Soil analysis shows the low of soil in organic matter, nitrogen, and phosphorus. These nutrients play an important role in soil fertility and improving agricultural production (4).

5.2 Effect of amendments on the size of Culture

We note that the size of each treatment culture are different from one plot to another, the average recorded in a period of two months is very highly significant compared to the control. It is more important in test I (10t/ha) of stable product at a value of 60.2 ± 0.27 cm, 62.1 ± 0.24 cm for the test of 15T/ha II, against the culture of potato amended by chemical fertilizer does not exceed 55.8 ± 0.24 cm for a period of four months.

5.3 Calculation of yield

Crop yields of potatoes produced ranged from 14.2 ± 0.27 and 17.8 ± 0.34 t/ha, with a maximum value of 17.8 ± 0.34 t / ha obtained in the case of test II (15T/ha).
Comparison of means allowed to gather the doses Test I (10t/ha) and Test II (15t/ha) in the same class. This suggests that the dose (15t/ha) allows to realize the maximum yield compared with the fertilizer that does not exceed a yield of 16.1 ± 0.41 t/ha.

5.4 Effects of studied products amendments on the soil

The maximum value of organic matter was obtained in the test of 15T/ha after removal of co-product after removal of culture of potato, it is between 3.61 ± 0.27 and 4.18 ± 0.19% , which shows that the applied dose of co-product has a highly significant effect on the chemical composition of the soil at the end of the vegetative cycle (13).
Level of total nitrogen in the soil after harvest varies with the type of treatment applied, and depending on the dose given. The total nitrogen content of the soil increased significantly (0.57 ± 0.27%) at a dose of 15T/ha, however, plots soil analysis 10t/ha (0.34 ± 0.15%) and chemical fertilizer showed a slight increase in nitrogen content (2).
For the content soil in phosphorus and potassium, we find that the contribution of co-product to soil has improved its content and reserves of phosphorus and potassium assimilated in an amazing way. Its reserves in

assimilated potassium were almost doubled by two doses made to reach values of 1.05 ± 0 .23% and 1.27 ± 0 .29% in the case of potato cultivation, while these phosphorus reserves were increased by an excessive way compared to the initial state (14).

It is important to note that the two trials (10t/ha and 15T/ha) of studied bio-fertilizers were characterized by a slight variation in their chemical composition with a greater value fertilizer on soil characteristics and on yields. Several authors (5) and (1) found a significant relationship between organic matter, nitrogen and dry matter content at the end of the vegetative cycle. This relationship may be due to the increased participation of nitrogen and sugary compounds and anions to maintain balance of osmotic pressure during the growth and development of plants (6).Bio-fertilizer obtained has a significant effect on soil characteristics. Soil analysis after the end of the vegetative cycle of potato showed that there is a very remarkable and important bio-fertilizers (15T/ha) on the chemical composition of the soil at the end of the cycle. It was found that the rate of organic matter in the soil receiving various products of amendments depends on the dose given and the date of sowing, in all cases, we observe an increase in the rate of organic matter compared to the initial state (10). In addition, we must not forget the fertilizer value attributed to residual effects of these amendments for the next crop. The chemical state of the soil evolved in total nitrogen and assimilable potassium for both doses of bio-fertilizer obtained (15).

6. Conclusion

By a biotransformation process using pure strains of lactic acid bacteria and yeast, we have shown that it is possible to transform slaughterhouses waste of red meat had a finished product of hygienic and organoleptic quality. This process seems a simple and effective stabilization for their recycling and recovery, and help to reduce the negative impact of waste on the environment.Regarding to chemical composition, slaughterhouse wastes are poor in nitrogen and their incorporation into rations will be accompanied by a reduction in their nitrogenous value. Therefore, these wastes can not be used effectively till if nitrogen supplementation is considered, particularly with a source of non-protein nitrogen. Subsequently, the stable product obtained has important characteristics namely the complete disappearance of the unpleasant odor characteristic of slaughterhouse wastes, and a considerable improvement in its hygienic quality resulted in a reduction of almost all the hygienic flora.

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Table 2: Characteristics of lactic acid bacteria grown in liquid MRS medium for 48 h at 30 ° C in the dark**.**

Biotope	**Strain**	**Final pH**	**Bactericidal Test**	**Kind**
Cow's milk	**BLh1**	4,45	+	*Lactococcus sp*
	BLh2	4,84	++	*Lactobacillus sp*
	BLh3	3,93	-	Indeterminate
	BLh4	4,23	-	indeterminate
Press juice of cane sugar	**BLh5**	3,75	+++	*Streptococcus sp*
	BLh6	4,40	++	indeterminate
	BLh7	4,04	+	indeterminate
	BLh10	3,86	+++	*Enterococcus sp*
	BLh11	4,12	+	*Enterococcus sp*
	BLh12	4,02	-	*Lactococcus sp*
	BLh13	3,82	-	indeterminate
	BLh14	4,16	-	*Streptococcus sp*
Press juice of cane sugar after liming	**BLh15**	4,08	+	*Lactobacillus sp*
	BLh16	3,98	+	*Lactobacillus sp*

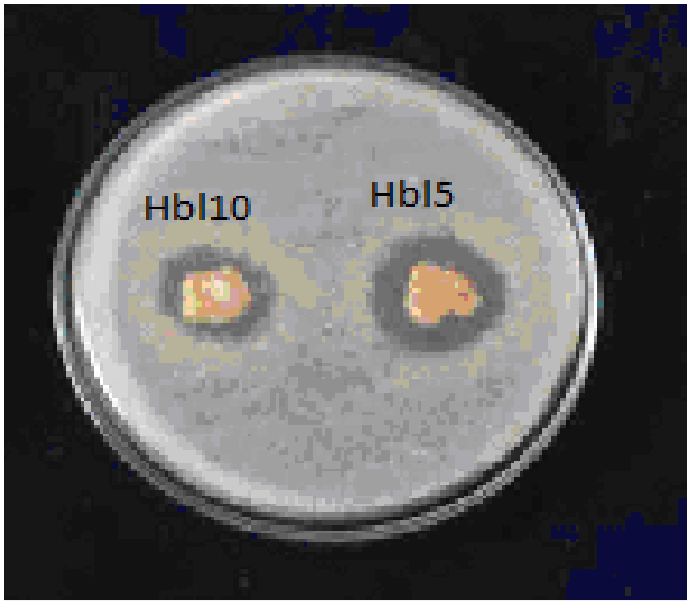

Figure 1: Evaluation of antibacterial activity of two lactic strains (HBL5 and HBL10) vis-à-vis Staphylococcus aureus ATCC 25923

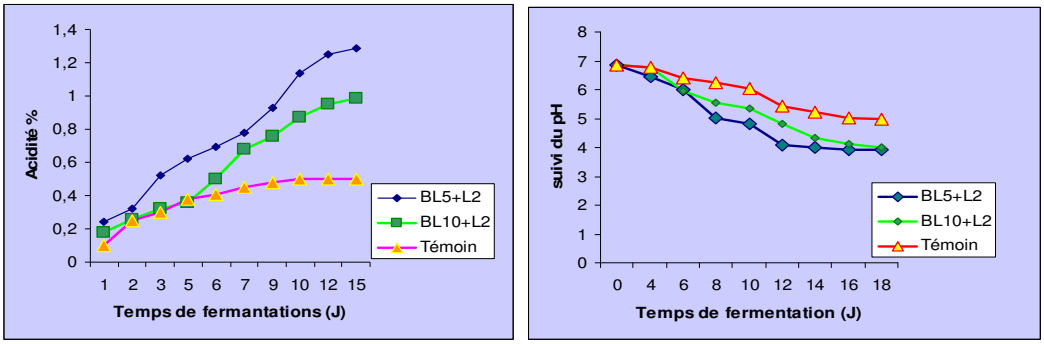

Figure 2: Evolution of pH and acidity during the fermentation of wastes inoculated by lactic strains with strain Lh2.

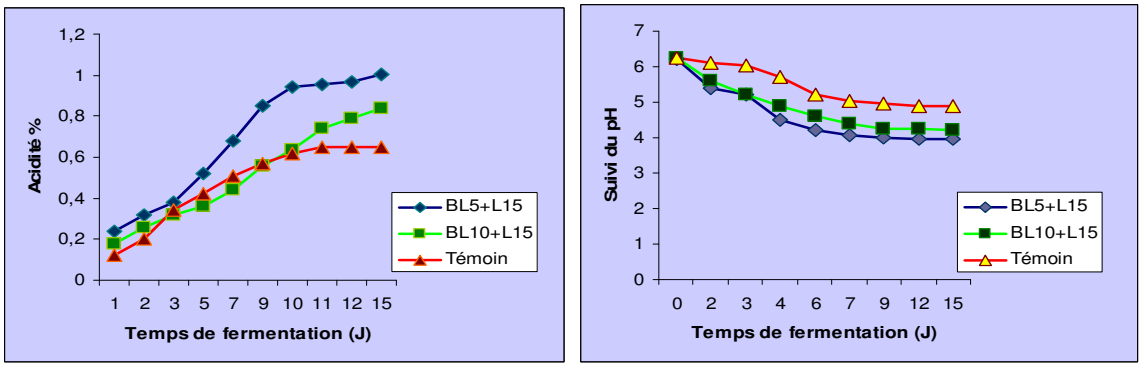

Figure 3: Evolution of pH and acidity during the fermentation waste inoculated by lactic strains with HL15.

Table 3: Physico-chemical analysis of the finished product after fermentation waste

Physico-chimical Parameters	**Initial Product**	**Finished Product**
pH	6,78	3,91
Titrable Acidity %	0,22	1,25
Total Nitrogen %	3,77	4,23
Organic Carbon %	15,42	21,02
Phosphorus $\%_0$	5,02	5,32
Potassium $\%_0$	37,25	38,03
Dry Matter %	32,11	33,98
Organic Matter %	43,58	40,17

Table 4: Microbiological analysis of finished product after fermentation waste

Microorganisms	**Initial Product (ufc/g)**	**Finished Product (ufc/g)**
MTAF	6.10^8	7.10^5
Lactic Bactéria	42.10^5	32.10^7
Yeasts	12.10^4	3.10^5
Fecal Coliforms	5.10^5	0
Salmonella	0	0
Fecal Streptococci	8.10^5	2.10^2
Clostridia	248	≤ 10
Staphylococci	7.10^4	0

Table 5: Physico-chemical analysis of the clay soil used before the preparation of potato cultivation.

Parameters	**Soil before cultivation**
Size	
Fine soil	100%
Clay	67,2%
Fine silt	27,3%
Coarse silt	4,7%
Fine sand	0.50%
Coarse sand	0,3%
chemical composition	
pH_{water}	7,54
Organic matter	2,31%
Total nitrogen	0,47%
Assimilable phosphorus	0,32%
Potassium	0,62%

Table 6: Evolution of potato crop size

Date of mesure	**Control (cm)**	**Fertilizer (cm)**	**Manure (cm)**	**Test I (cm)**	**Test II (cm)**
10/11/10	06.5± 0,45	07,8± 0,12	07,8± 0,12	07,6± 0,24	08,2± 0,11
25/11/10	12,4± 0,37	14,7± 0,27	15,2± 0,17	14,2± 0,19	16,04± 0,21
10/12/10	15,7± 0,27	20,4± 0.18	21,1± 0,31	20,5± 0,33	22,7± 0,27
25/12/10	21,5± 0,41	24.2± 0.27	25,8± 0,27	23,8± 0,17	26,7± 0,19
10/01/11	27,4± 0,33	31.4± 0,31	33,2± 0,23	32,1± 0,21	34,7± 0,23
25/01/11	35,3± 0,17	38.1± 0.25	39,7± 0,19	38,2± 0,32	40,1± 0,14
10/02/11	41,5± 0,18	45.8± 0.19	47,2± 0,21	48,5± 0,14	48.6± 0,17
25/02/11	47.1± 0,31	50.2± 0.27	54,6± 0,33	54.7± 0,19	55,2± 0,22
10/03/11	49.2± 0,33	55.8± 0.24	60,7± 0,.18	60.2± 0,27	62,1± 0,.24

Table 7: Crop yields of potatoes for the applied amendments

Plot	**Number of tubers / plant**	**Tuber weight (average)**	**Number of stems /m²**	**Yield (T/ha)**
Control	7± 0,17	48± 0,23	14± 0,17	14,2± 0,27
Chemical fertilizer	9± 0,23	56± 0,19	17± 0,22	16,1± 0,41
manure	10± 0,23	64± 0,27	18± 0,27	17,2± 0,23
Test I (10T/ha)	9± 0,27	58± 0,33	16± 0,23	16,4± 0,33
Test II (15T/ha)	12± 0,34	70± 0,25	18± 0,25	17,8± 0,34

Table 8: Effects of studied amendments products on soil chemical properties after the potato crop

Traitement	**Organic Mater** %	Total Nitrogen %	Phosphorus%	Potassium%
Control	1,02 ± 0,17	0,21 ± 0,18	0,023 ± 0,11	0,057 ± 0,23
Chemical fertilizer	1,12 ± 0,23	0,43 ± 0,22	1,11± 0,17	1,07 ± 0,27
manure (25t/ha)	4,22± 0,19	0,51± 0,24	0,47± 0,23	0,71± 0,18
Test I (10T/ha)	3,61 ± 0,27	0,34 ± 0,15	1,02 ± 0,26	1,05 ± 0,.23
Test II (15T/ha)	4,18 ± 0,19	0,57 ± 0,27	1,15 ± 0,18	1,27 ± 0,.29