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RESEARCH ARTICLE

Characterization, treatment and recovery of fish by-product as a stable bio-fertilizer

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ABSTRACT: National fish production reached in 2013 an amount of 1,245,912 tons. Now the pelagic fish industry generates a significant amount of waste up to 60%. The physicochemical analyses of these fish by-products showed a wealth of organic material including proteins (87.4% dry weight) and minerals (9% dry weight). These components and others are capitalizing to be used for agricultural or agri-food. Biotransformation of biotechnologically in fish products was performed in the laboratory. Fresh fish by-products were ground and mixed with a carbon-rich source of carbohydrates. The mixture was inoculated with selected fermentation yeast. The fermentation of evolution was controlled by monitoring chemical parameters (pH, dry matter, ash, total nitrogen) and microbiological (FMAT, *Enterobacteriaceae*: fecal and total *Coliforms*, yeasts). Then the product thus stabilized at a pH of 3.8 has undergone a second treatment. A hydrolyzate rich in amino acids and trace elements was obtained with excellent hygienic quality after a total elimination of pathogens. Its use will be as factors for fertilizer and soil amendment. We produced three biostimulants necessary for plant growth: Rooting fertilizer, Plant elongation fertilizer and production fertilizer. The application tests on these biofertilizers in the laboratory were performed and have allowed obtaining promising results.
Keywords: biotransformation, fermentation, fish by-products, fertilizer, hydrolyzate, fertilizers and biostimulants.

INTRODUCTION

The geographical position of Morocco between two great oceans Atlantic and Mediterranean has made him a major source of fish production and its derivatives. Indeed, the national fish production reached in 2013 an amount of 1,245,912 tons (Anonymous, 2013). The structure of national fish production is still dominated by small-scale coastal fishing (71%). The offshore fishery continues to strengthen its contribution with 21% of tonnage and 38% of the value (Anonymous, 2011).

The processing industry and exploitation of fishery products in Morocco occupy a privileged place in the national economy because it handles nearly 70% of catches of inshore fishing and exports about 85% of its production on a hundred countries in five continents. Otherwise; the pelagic fish industry generates a significant amount of waste up to 60%. Indeed, the quantities of fish by products (fish offal and fish not machinable and unprocessed) are estimated at several hundred thousand tons of waste per year (Chabbar AZ., 1996; Afilal M.E *et al*, 2014). These wastes like other residues are often deposited in landfills without prior treatment with negative consequences both on the health of the population bordering the presence of pathogens and the environment.

However, the physicochemical analyses of these fish by-products showed a wealth of organic material including proteins (87.4% dry weight) and minerals (9% dry weight). These components and others are capitalizing to use for agriculture or food processing. Hence the need for the establishment of an industrial process of their treatments and their valuations. Our present project is part of the concern. It is based on the operation of an industrial process for processing, transformation and exploitation of these fish by-products by biotechnological ways that are the subject of an invention patent (Patent Number 24649). A simple process inexpensive and easily applicable which enabled obtaining liquid organic fertilizer rich in amino acids and trace elements to bring the different elements necessary for the development of vegetable crops.

Our work has focused on the use of these products as bio-stimulants of plant growth. They possess unique biological and physico-chemical properties that activate metabolic and physiological processes of the plant. In effect, three types of bio-stimulants were prepared and tested: bio-stimulant root development, bio-stimulant elongation of the plant and bio-stimulant production.
The application tests have allowed obtaining promising results.

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MATERIAL AND METHODS

2.1. Sampling

Samples in fish products were collected at various outlets in the city of Kenitra, they are composed of different parts of fish: head, chef, viscera, stop, spines and sometimes whole fish. The most dominant species in the composition of these samples is Moroccan sardine: *Sardina pilchardus*. These samples are immediately transported to the laboratory for physicochemical and microbiological analyses.

2.2. Physico-chemical and microbiological testing of fish by-products

2.2.1. Crushing

The samples immediately arrived at the lab suffered a crushing under sterile conditions with a device of Moulinex "roping xxl picadora 1, 2.3" of French manufacture.

2.2.2. PHYSICO-CHEMICAL ANALYSES

2.2.2.1. Sample temperature

We worked under laboratory conditions at room temperature 20 ° C.

2.2.2.2. pH measurement

The pH of each test is measured daily using a pH meter (type Orion Reseach). Its calibration at pH 4 and 7 is carried out before any measurement. The values are taken directly from the display cell.

2.2.2.3. Acidity Dosage

The acidity is measured by titration of filtrate 10ml with an alkaline solution (NaOH) N/9 in the presence of phenolphthalein as a color indicator. The acidity is expressed as % of lactic acid. The results are expressed according to the following equation:

$$\textbf{Acidity \%} = \frac{\text{Vol (NaOH). (N NaOH). (Volume taken). (100)}}{\text{(Sample mass)}}$$

2.2.2.4. Determination of the dry matter

The dry matter is determined by baking a quantity of fish by-product at 105°C overnight (Lovergrove Y., 1966). This is to complete a previously dried and weighed crucible by heating in the oven and cooling on a sample of fish product.

2.2.2.5. Determination of Moisture

The humidity is the percentage of water component in a sample of the product fresh fish. The technique for achieving this operation is the same as described for the determination of the dry matter.

2.2.2.6. Determination of ash

These ashes are obtained from the sample already dried by incineration at 550°C in an oven for 4 hours until white or gray ash.

2.2.2.7. Determination of total sugar content

The total sugars were determined by the phenol method (Dubois, 1952).

2.2.2.8. Determination of reducing sugars

The free reducing sugars are assayed by the method of Somogyi (1951) and Nelson (1944) by measuring the absorbance at 540 nm. The calibration curve is produced under the same assay conditions using a solution of 0.3g / L glucose + 0.3 g / l fructose.

2.2.2.9. Determination of total nitrogen (Kjeldahl method)

Total nitrogen is determined by the Kjeldahl method (Bradstre et R.B, 1965). It is determined by mineralization with concentrated sulfuric acid in the presence of catalysts. The reaction product ammonium sulphate $(NH4)_2SO4$ is moved by a boric acid and titrated by hydrochloric acid.

2.2.2.10. Total protein

The amount of protein is calculated based on the total nitrogen content of the sample using the following formula: (Protein) = 6.25. N (%).

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2.2.2.11. Fat (Soxhlet method)

The fat sample was extracted with a polar solvent (ethanol). The solvent to boiling rises to the porous cartridge, and back, it causes the fat therein. The rate minerals are determined by spectrophotometric assay according to French standard NF V18-106 (AFNOR, NF V18-106, 1980).

2.2.3. Microbiological analyses of fish by-product

2.2.3.1. Flora sanitary interest

Enumeration of Mesophilic Aerobic Total Flora (FMAT): Counting of FMAT was performed on medium Plate Count Agar (PCA) by seeding depth of 1 ml dilutions ranging from 10-1 to 10-7. It is used reading is taken after 48 hours of incubation at 30°C.

2.2.3.2. Enumeration of *coliforms*

The total coliform was performed on MacConkey medium to visualize the presence of *coliforms* as a hygiene indicator (Zahar M. *et al.*, 2002). The cultures are incubated at 37°C for 48h. Fecal *coliforms* are incubated in the same medium at 44°C.

2.2.4. Pathogenic and toxigenic flora

2.2.4.1. Enumeration of *Staphylococci*

Staphylococci are known for their high resistance to salts. In this sense, the enumeration of *staphylococci* is performed on selective medium Chapman or Mannitol Salt Agar medium containing 75% NaCl (Branger A. *et al.*, 2007). The cultures are incubated at 37°C for 24 hours.

2.2.4.2. Faecal *streptococci* enumeration

Fecal *streptococci* are counted on Sodium azide selective medium. Incubation is performed at 37°C for 24 hours.

2.2.4.3. Detection of *Salmonella-Shiguella*

It is made by inoculating 1 ml of pre-enrichment medium in 9ml of a selective medium broth tetrathionate or selenite cysteine broth (BSC). After homogenizing, the tubes were incubated at 44°C for 24 hours for the first and pending 24h at 37°C for the second.

2.2.4.4. Enumeration of sulphite-reducing Clostridia

The Reinforced Clostridial medium Agar (RCA) contains cysteine as the reducing agent, its degradation by *Clostridium* releases sulfur which combines with the iron to thereby give a black color in the colonies of *Clostridium*. After 48 hours of incubation at 37 °C, we perform a reading to the individualized black colonies at the bottom of the tubes after incubation at 37 °C for 48 h.

2.2.4.5. Enumeration of proteolytic

Several media can be used to quantify the proteolytic flora. In our case we used to count these microorganisms, the nutrient agar solid casein. Hydrolysis of casein was studied in medium composed of PCA nutrient agar supplemented with 50% skimmed milk. The medium is poured into Petri dishes after 1 ml seeded dilution of 10-1 to 10-5. After 3 days of incubation at 30°C, colonies hydrolyzing casein have a clear halo around the colony.

2.2.4.6. Lactic acid bacteria

Lactic acid bacteria were counted on the middle of Man, Rogosa and Sharpe (MRS). The dilutions of 10-4 to 10-7 are seeded in depth. Incubation is performed at 30°C for 48 hours and only those colonies having an appropriate shape are retained.

2.2.4.7. The yeast

Yeasts are listed on the solid medium of Saboraud chloramphenicol. 0.1 ml of the sample was inoculated and incubated at 30°C for 48h. The colonies are selected.

2.3. Salaries of fish by product

80 Kg of fish by-products contain an added carbon source at 20% in barrels. Each barrel is inoculated with yeast fermentation at 0.6 g/l compound strains of bacteria with a fermentative, acid and bacterial power isolated from different habitats stored at the laboratory level (Labioui H. *et al.*, 2006). Control and monitoring of the fermentation at room temperature are carried out by pH measurement until stabilization of the product at a constant pH.

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2.3.1. Application Essay: culture in the laboratory

2.3.1.1. Plant Material and Culture Conditions

In the selection of crops that will be studying the effect of these bio-fertilizers on their different development and productivity criteria were taken into account mainly including the importance of the area planted annually by the culture and socio-economic role in Morocco. Thus, the choice was focused on Corn seeing that it is a dual-purpose crop (animal and human consumption) that comes after cereals in terms of economic importance and also for its role in the dairy sector as food basic after silage for dairy cows and as seeds for the production of white meat in poultry farming. In addition, the corn crop in Morocco confirms the adaptability of this plant to modern technical lines (micro irrigation, tillage, fertigation) and sensitivity to organic fertilizers including bio stimulants and humic substances.

2.3.1.2. Floor selection

The soil with which we worked is taken from the entourage of the faculty. It is a poor sandy soil in minerals that have benefited from any fertilizer material inputs or fertilizers, manure or compost. Watering of the cultures was taken. Seeds: the application tests were conducted on corn cultivation.

2.3.1.3. Bio-stimulants

The Bio-stimulants were provided by the SINVA Company - Morocco whose characteristics and composition are indicated on the packaging of each labeled Bio-stimulants.

2.3.1.3.1. Bio-stimulating rooting: Composition (Table 1)

Its application is performed by injecting an amount of 5 to 7 liters / hectare for market gardening and 7 to 9 liters / ha for Citrus and fruit trees.

2.3.1.3.2. Bio-stimulant elongation-production: Composition (Table 2)

Marketed under the name ALFAMINE, it is also a liquid product containing free amino acids. It can be applied at any time of the growing season. Its application is by foliar and irrigation water. Applying foliar Citrus tree at 5 to 7 liters / hectare for market gardening and 7 to 9 liters / ha for Citrus and fruit trees.

2.3.2. Application

Initially, and after the filling of pots with soil, it performed its irrigation with pure water until its field capacity.
In parallel, and in an objective to ensure homogenization and a high germination rate, corn seeds are placed on wet filter paper and germinated in the dark in an incubator at 28 °C, relative humidity of 100% for 5 days. Young seedlings are then put in pots with respect dimensions: The sowing operation, the dose and the spacing between line and applied seed as shown below in Fig. 1 - (Control and test of the same size and in the same conditions). Each pot is provided with a label indicating the name of culture. Each pot contains the same amount and the same soil type. It should be noted that the pots are drilled at the bottom to allow drainage of excess water during watering crops. 10 days after sowing, we began the implementation of bio stimulating rooting. The contributions were made by direct watering every 10 days in 3 periods. Monitoring the development and evolution of the roots were performed by a weekly levy roots with 3 samples taken. The evaluation indicators taken into account are the size and weight of the roots and the comments made on the overall appearance of roots. The same technique was performed to the stimulus of elongation and development and fruiting by foliar application by measuring the size, the weight of the stem, leaves and fruit.

RESULTS AND DISCUSSION

3.1. Study physicochemical and microbiological testing of fish by-product

3.1.1. Physical and chemical properties

The results of the physicochemical analyses of the front sub processing fish products showed that the pH of the fish by-product is 5.9 and a humidity of 70% (Table 3). The dry matter is 34.12%. These results are in agreement with the work of Zarembinski (Rahmi M. *et al*., 2008). With regard to the protein material, the fish by-products show a significant wealth with a protein content of 26g.
Furthermore, the composition:

- Elements macros: Nitrogen (N) is estimated at 11.2%, phosphorus (P) 450 mg potassium (K) to 390 mg.
- Secondary elements: Calcium (Ca) is estimated at 375 mg, magnesium (Mg) to 40 mg.
- Trace elements: Iron (Fe) is estimated 3.2 mg, manganese (Mn) to 0.110 mg, zinc (Zn) 1.4 mg, 0.200 mg copper.

Given the results achieved and the by-products as well show richness in organic and mineral matter constituting a qualitative and quantitative potential for possible development and exploitation in agricultural production.

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3.1.2. Microbiological Study

The results of microbiological analyses performed on 10 samples of sub treatments before fish products are summarized in Table 4. These results show that in fish products are home to several pathogenic bacterial species. The Total Aerobic *Mesophilic Flora* (FMAT) showed significant bio-burden estimated to average 1.6 108 CFU / g, total *coliforms* 3.6 105 CFU / g, *Staphylococci* of 3.7104 CFU / g, Sulphite-reducing *Clostridium* 494 CFU /g, Faecal *Streptococci* and 120 CFU / g. This microbial burden is a serious environmental problem and public health, hence the need for an effective and sustainable processing of fish by-products. In the present work we have opted for a biological treatment for a yeast fermentation performance prepared from selected strains formed in laboratory bacteria with a high fermentative, acidifying and antibacterial power.

3.2. The processing steps in the fish product

The biological conversion process of fish by-product developed in the laboratory with a view to obtaining a hydrolyzate rich in amino acids. This technique proves original, fast and results in the formation of substances that are components of various bio-stimulants liquids and the synthesis process has undergone many developments. Technology transfer, in collaboration with the SINVA Company - Morocco specialized in the manufacture of fertilizers and fertilizer. Treating biologically fish by-products produced according to the steps illustrated in Fig. 2. To this end, 80 kg of fish by-products contain an added carbon source at 20% in barrels. Each barrel is inoculated with yeast fermentation at a rate of 0.6 g/l at room temperature. The control and monitoring of fermentation are made by measuring pH and the evolution of pre-existing pathogenic microbial flora and the evolution of bacterial strains of the yeast fermentation.

3.3. Physicochemical and microbiological characterization in fish products after processing and stabilization

The analyses of the physicochemical parameters of the stabilized product obtained show that the initial pH of 5,9 decreases gradually during the fermentation to stabilize at pH = 3.5 (Table 5). The inoculum accelerated the fermentation process and has reduced or eliminated the lag phase, which can take two days or more under normal conditions. This reduction of pH in the fermentation product reveals a good acidification, and consequently a good stabilization and storage of the fermented product. This pH drop is attributed to leaven that by consuming fermentable sugars contributed by the carbon source, producing organic acids (lactic acid and/or acetic acid) responsible for the lowering of the pH and thus contributing to the preservation of fish waste (Vibeke, 1993). A slight decrease of dry matter, up from 48.72% in the initial product at 45.32%. The same trends are obtained by Chouikhi (Faid M. *et al.*, 1997), for other waste. This decrease is probably caused by their use of the microorganisms and the production of volatile components during the fermentation process. Although the work carried out by Haaland (Haaland H. & LR. Njaa. 1990), showed that there is increase in dry matter and that this increase may be due to the loss of water by evaporation or water binding proteins during autolysis and probably also triglycerides. A third hypothesis suggests that the increase in the dry matter is attributed to a loss of carbon dioxide and ethanol (by evaporation) during fermentation.

The other parameters (MAT and ash) show a slight increase during the fermentation process. Ash rate rose from 6.95% in the initial product to 7.71% in the finished product. The same trends are obtained by Hammoumi (Hammoumi, A. *et al.*, 1999). The increase of non-protein nitrogen is mainly due to enzymatic proteolysis phenomena while that of total nitrogen and ash is due to production of volatile substances and evaporation of water. Regarding minerals and trace elements, we have not found a significant difference. Moreover, the protein also showed a slight decrease from 20.8% to 19.25%. This result is consistent with previous work that reported a decrease in protein levels of the fermentation process and the decrease by liquefaction proteins that can lead to a loss of nitrogen as ammonia (Faid M. *et al.*, 1997). The non-protein nitrogen has been increased from 4.25% in the initial product to 6.31% of the finished product. Hammoumi find very different values of 9.74% in the initial product and 31.68% of the finished product (Hammoumi, A. *et al.*, 1999).

The wealth of amino acids in this product is not only quantitative but it is also qualitative. Indeed the fermented product contains the different ranges of amino acids, including essential amino acids (lysine and methionine) (Table 6). The total wealth of nitrogen and non-protein nitrogen, after the stabilization of the finished product are significantly higher, showing the richness of these by-products in crude protein and amino acids; hence, their interest in their use as ingredient in a complex formulation of bio-stimulants containing amino acids to ensure the plant has a rapid and balanced nutritional intake.

3.4. Microbiological analysis of the product under in biological treatment processes

Microbiological analyses during fermentation are reported in Table 7. These analyses show that:
During the fermentation the FMAT suffered a significant decline. It spends 1, 6.108 ufc/g. 6, 1.105 to cfu / g at the end of fermentation. This is the main result of a decrease in pH due to increased production of organic acids including lactic acid.

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Unwanted microflora is the only constraint to watch in a stabilization process. This microflora is divided into two major categories, pathogens and toxigenic species and alteration species. The former are involved in food poisoning and the latter alter the product with production of volatile compounds that are causing noxious odors. The evolution of hygienic interest microflora was followed during the fermentation of fish by-products, the results show that during the first phase of fermentation, hygienic interest microorganisms increases slightly because the medium pH is close to neutrality. This phase corresponds to the acidifying bacteria lag phase. Then during the second phase (3rd to 7th day) fermentation, there is a significant decrease. A virtual elimination is observed during the third phase of fermentation (>7 days). Indeed during the fermentation charge in total *coliforms*, fecal and *Clostridia* rose respectively 3, 6.105, 1, 1.103 and 5.102 CFU / g at the start of fermentation to an almost complete disappearance of these germs in late fermentation. This demonstrates the efficiency of fermentation processing. This decrease is mainly due to acidifying and fermentative power of yeast fermentation and antibacterial activity. Indeed flora has fermentative and acidifying power which spent 3,2.106 to 4,8.109 CFU/g (Labioui H *et al.*, 2006), Sourdough fermentation has ensured in the fermentation of fish products under the presence of a concentration of a carbon source. The product is stabilized at a pH of 3.5 with inhibition of nematodes and disappearance of unpleasant odors and the total disappearance of pathogens and toxigenic at the end of fermentation. This shows the effectiveness of our method and biotechnological interest for our product. This result is consistent with previous work Rahmi M. *et al.*, 2008; Faid M. *et al.*, 1997).
The pH drop in the fermentation system is an important factor for stabilization and processing under rich fish products in organic matter (Haaland H. *et al.*, 1990, Faid M. *et al.*, 1994, Faid M. *et al.*, 1995), and it is a quality closely related to the growth of lactic acid bacteria. This property is desired in the case of biotransformation (Faid M. *et al.*, 1994, Faid M. *et al.*, 1995). Decreasing pH and increasing the acidity reflect the degree of hydrolysis of the carbohydrate substrates.

3.5. Product recovery test obtained after stabilization by fermentation

The chemical characterization of the finished product after biotransformation and stabilization showed a wealth of nutrients including high content of organic matter with a potassium superiority and nitrogen and rich in trace elements and amino acids. These features meet the standards of fertilizer applied in the field of fertilizers to improve agricultural crops as it was recommended by Irmak (Irmak, S. *et al.*, 2000).The formulation of bio-stimulant to apply was conducted based on the cultural stage of development (rooting-development and elongation-production) with a dosage of 3.5 liters of bio-stimulant in 100 liters of water). The results effects of the contribution of bio-stimulant tests on the growth cycle of corn were evaluated over a period of 3 months.

3.6. Effect of applying bio-stimulant on root growth

This is essentially a liquid containing amino acids in combination with polysaccharides, phosphorus and other essential trace elements to ensure the crop a rapid and balanced nutritional intake. The maize plants are planted in pots and watered with the bio-stimulating root solution once a week for the duration of the culture. At each sampling, the roots are washed, weighed and photographed. Root biomass compared to biomass of control plants (grown without the use of molecules). The overall results are presented in the photo 1 and Fig. 3. A dramatic increase in root biomass of maize seedlings treated with the extract of bio-stimulating rooting and roots in implementation compared to the control untreated seedlings, in fact it goes from 0.48g to 750g after 90 days of culture, while the indicator did not exceed 150g. In addition, it is found that this increase in root biomass is accompanied by a change in root architecture, including an increase in the number of secondary roots an increased root length and associated with a lateral root development.

3.7. Effect of bio-stimulant application on the growth of the aerial part

To highlight the role of bio-stimulant on the aerial part of the plant we followed the evolution of the size of the diameter and leaf area during the vegetative cycle.

3.8. Effect on the culture of size

The results for the response of the culture of corn to bio-stimulants elongation (Fig. 4), show that there is a highly significant effect compared to the control of the evolution of the size of the crops grown in growth cycle, it goes from 47cm to 25 cm against the witness after 26 days of cultures to 225 cm against 150 cm for the control after 3 months of the growing season.
This shows that the bio-stimulant based on amino acids increases photosynthesis, to extend the size of the plant during the growing season and thus stimulates and increases plant resistance to stress conditions. It also stimulates metabolism and improves the general condition of the plant.

3.9. Effect on diameter and leaf area of the plant during the vegetative Maize cycle

The results of the tests (Table 8) show highly significant effect for both stem diameter leaf area compared with the control, it passes to 4.35 cm diameter value after one month of culture, reaching a maximum value 8.35cm after 3 months of the growing season against 5.35cm for the control after 3 months of the cycle. The same observation was made for the leaf surface. At the stage of leaf

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development, we are witnessing a growth of vegetation cover in all plants. A clear superiority was observed in plants that have undergone treatments with bio-stimulant. The value of the leaf area 24.15cm^2 obtained in the first month of growth cycle of corn rose to a maximum value of 71.27cm^2 against a value of 49.73 cm^2 for the control growth after 3 months.

We can conclude that the beneficial effects of extracts of bio-stimulant on root and leaf biomass plant would be for part due to the action of the nutrients provided by the extract. Alternatively, the effects of nutrients can be the cause of these changes respectively (N, P, K, Fe), trace elements, amino acids, polyamines, peptides and proteins on the growth of the main root and initiation of secondary roots and root hairs, these responses can be systemic or localized to a part of the roots. In this sense, the authors proposed two modes of action of nutrients on plant growth. Contribution they make induces a systemic response, i.e. an overall change in the plant development; the nutrients provided to the root level are absorbed then assimilated by the plant which modifies its nutritional status. This improvement of the nutritional status is perceived by the plant that changes throughout its development accordingly: increased root and leaf growth. Or, the nutrients cause localized response, that is to say a change in root architecture. Perception of the root level of the modification of the medium in the nutrient concentration resulting in the induction of signals leads to local modifications of the root and leaf architecture to optimize nutrient uptake. These localized responses can result in increases in root growth and root diameter as well as increases the number of secondary roots. (Forde B. *et al.*, 2001; Mugnai S. *et al.*, 2008). This modification occurs not only in the number of secondary roots, but also in the number of hairs. The root surface in contact with the medium thereby is greatly increased for the treated plants (Spinelli F. *et al.*, 2010; Eyheraguibel B. *et al.*, 2008; Mora V. *et al.*, 2010). Other authors have observed a significant relationship between the nitrogen content of the plant, fluid intake and plant growth; that is to say, the content of nitrogen removed by the plant increases the diameter profile of the plant during the growing season (Spinelli F. *et al.*, 2010; Roussos PA. *et al.*, 2009). This relationship explains the values recorded during measurement corn plant in diameter to achieve maximum value 9.25 cm and leaf area with 71.27cm^2 value. Furthermore, in several studies, this increase in aboveground biomass is accompanied by an increase in stomata aperture and the chlorophyll content of leaves. Furthermore, we have carried out extensive studies on growth, chlorophyll content and nitrogen use efficiency, transcriptomics analyses and physiological characterization of metabolism and the testing of large-scale cultivation in the fields will be the subject of two other articles under realization.

CONCLUSION

The results of the physico-chemical and microbiological analyses show that in fish products are loaded with pathogens and toxigenic including fecal *coliforms*, *Staphylococcus Aureus* and *Clostridium*. This constitutes a serious problem for public health and a negative impact on environment. Sustainable and effective solution is needed. The overall chemical composition of fish by-products highlights their potential nutritional value and hence the interest in their use as recoverable ingredient. In this perspective, we have identified a biological treatment process for waste based primarily on the use of sourdough made from microorganisms that have strong fermentative power, acidifying and antibacterial and allow the presence of a carbon source to stabilize these by-products and eliminate pathogenic microflora, toxigenic and spoilage, from which emanates the idea of their value and their use as bio-stimulant plant growth. They have biological properties and physicochemical exceptional chemical that activate metabolic and physiological processes of the plant. In fact, two types of bio-stimulants were formulated and tested based on the stage of crop development (rooting-development and elongation-production). Application tests conducted on these bio-fertilizers laboratory level allowed obtaining promising results and significant differences in terms of the size, leaf area and diameter of different plant organs were observed. This proves that the studied bio-stimulants have significant capacity to supply nitrogen mineral and organic material to the plant and the ground; therefore, to conclude that the bio-fertilizer used, once incorporated into the soil, is a valuable alternative as an amendment product. Testing of large-scale cultivation in the fields has been made. The results are highly significant will be the subject of future articles.

REFERENCES

1. Anonymous, 2013. La mer en chiffre, Ministère de l'agriculture et de la pêche maritime, département de La Pêche Maritime, Rabat.
2. Anonymous, 2011. La mer en chiffre, Ministère de l'agriculture et de la pêche maritime, département de La Pêche Maritime, Rabat.
3. Chabbar AZ., 1996, Technologie de l'ensilage de poisson et disponibilité de la matière première au Maroc. Mémoire de 3ème cycle, IAV Hassan II, Rabat, Maroc.
4. Afilal M.E., O. Elasri, Z. Merza 2014. Caractérisations des déchets organiques et évaluation du potentiel Biogaz (Organic waste characterization and evaluation of its potential biogas). J. Mater. Environ. Sci. N° 5 (4) pp : 1160-1169.

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http://dx.doi.org/10.22573/spg.ijals.017.s12200081

Available online at https://www.skyfox.co
ISSN: 2454-6127

5. Lovergrove Y., 1966. The determination of the dry weight of plankton and the effect of various factors on the values obtained. Sorne Contemporary Studies in Marine Science (Harold Bames Edo). George Allen and Unwin Ltd, London, pp: 429-467
6. Bradstre et R.B, 1965. The Kjeldahl Method for Organic Nitrogen, NY: Academic Press Incorporated, New York, pp: 39-88
7. AFNOR, NF V18-106, 1980. Aliments et produits animaux : Dosage des éléments minéraux, méthode spectrophotométrique.Association française de normalisation, Paris
8. Zahar M., N.Benkerroum, A. Guerouali, S.Baou, L.Alahiane.2002, Biological ensiling of sardine wastes in sugarcane molasse for their valorisation in animal feeding: microbiological study, Proceedings of International Symposium on Environmental Pollution Control and Waste Management, Tunis (EPCOWM'2002), Tunisie, pp: 304-311.
9. Branger A., Richer M. M., Roustel S., 2007. Microbiochimie *et al*imentation, Educagri Editions, 343 p.
10. Labioui H., Elmoualdi L., Ouhsine M, et Elyachioui M. 2006, Essai de valorisation des déchets des abattoirs comme un stable bio-engrais Journal Africain des Sciences de l'Environnement 1, 40-52
11. Rahmi M, M. Faid M, M. ElYachioui, E. Berny1, M.Fakir and M.Ouhssine (2008). Protein rich ingredients from fish waste for sheep feeding. African Journal of Microbiology Research Vol.(2) pp.073-077.
12. Vibeke, 1993. Biological preservation of sea food by lactic acid bacteria, Info fish international 5: 29-34.
13. Faid M , Zouiten, El Marrakchi and Achkari-Begdouri, 1997, Biotransformation of fish waste into stable feed ingredient, Food Chemistry, 60, 1, pp.13-18.
14. Haaland H., LR. Njaa. 1990. Fish silages prepared from raw materials of varying quality. Chemical analysis related to balance experiments in rats. - Fisheridinektorates Skrifter Serie Ernaeing 3, P 27-35.
15. Haaland H., Espe M., Njaa L.R. & Myklestad H., 1990, Chemical composition and variation in some parameters during storage of 8 formic silages prepared from capelin.Fisk. Dir. Skr. Ser.Ernoering. Vol III (2) : 59-74
16. Hammoumi, A., Faïd, A., & Amarouch, H. (1999). Utilisation des déchets de poisson fermentés par voie biotechnologique en alimentation animale. Cahiers Agricultures, 8(3), 207-209.
17. Hammoumi, S., Payen, A., Favre, J. D., Balmes, J. L., Benard, J. Y., Husson, M., ... & Daoust, M. (1999). Does the short variant of the serotonin transporter linked polymorphic region constitute a marker of alcohol dependence?. Alcohol, 17(2), 107-112.
18. Faid M., H. Karani, A. Elmarrakchi, A. Achkari-Begdouri. 1994. A Biotechnological Process For The Valorization Of Fish Waste. Bio/Technol : 49.P 237-241.
19. Faid M., H. Karani, A. Elmarrakchi, A. Achkari-Begdouri. . 1995. Transformation Des Déchets De Poisson Par Voie Biotechnologique, Cah. Agric 4 .P109-112.
20. Irmak, S., Haman, D. Z., and Bastug, R. 2000. Determination of crop water stress index for irrigation timing and yield estimation of corn. Agron J , Vol. 92, No. 6, pp. 1221-1227.
21. Forde B, Lorenzo H (2001) The nutritional control of root development. Plant and Soil 232:51-68.
22. Mugnai S, Azzarello E, Pandolfi C, Salamagne S, Briand X, Mancuso S (2008). Enhancement of ammonium and potassium root influxes by the application of marine bioactive substances positively affects Vitis vinifera plant growth. Journal of Applied Phycology 20: 177-182
23. Spinelli F, Fiori G, Noferini M, Sprocatti M, Costa G (2010) A novel type of seaweed extract as a natural alternative to the use of iron chelates in strawberry production. Scientia Horticulturae 125: 263-269.
24. Eyheraguibel B, Silvestre J, Morard P (2008) Effects of humic substances derived from organic waste enhancement on the growth and mineral nutrition of maize. Bioresource Technology 99: 4206-4212.
25. Mora V, Bacaicoa E, Zamarreno AM, Aguirre E, Garnica M, Fuentes M, Garcia-Mina JM (2010) Action of humic acid on promotion of cucumber shoot growth involves related changes associated with the root-to-shoot distribution of cytokinins, polyamines and mineral nutrients. Journal of Plant Physiology 167: 633-642.
26. Roussos PA, Denaka NK, Damvakaris T (2009) Strawberry fruit quality attributes afterapplication of plant growth stimulating compounds. Scientia Horticulturae 119: 138-146.

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Table 1: Composition (Bio-stimulating rooting)

Composition	% (p/v)
Free amino acids	8,0
total nitrogen	17,5
urea nitrogen	9,0
ammonia nitrogen	3,0
Phosphorus (P2O5)	3,5
Potassium Oxide (K2O)	3,0
polysaccharides	3,0
Density = 1,2	***pH=2,5***

Table 2: Composition (Bio-stimulant elongation-production)

Composition	% (p/v)
total amino acids	30,00
Free amino acids	2,00
total nitrogen	16,8
urea nitrogen	7,0
ammonia nitrogen	2,0
Potassium Oxide (K2O)	3,0
Density = 1,2	***pH=2,5 à 4***

Table 3: Physical and chemical analyses of fish by-products before treatment.

pH		5,9
Humidity		70,21
Dry matter (%)		34,12
Total nitrogen (%)		11,2
protein		26g
lipids		9,6g
total sugar		00g
Water		60 g
Minerals and trace elements	**Potassium**	390 mg
	Phosphorus	450 mg
	Calcium	375 mg
	Sodium	460 mg
	Magnesium	40 mg
	Iron	3.2 mg
	Zinc	1,4 mg
	Copper	0,200 mg
	Manganese	0,110 mg
	Selenium	52,7µg

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Table 4: Microbiological analyzes of fish by-products before processing.

Germs / Sample	FMAT. ufc/g	Colif. ufc/g	*Staphy.* ufc/g	*Strep.* ufc/g	*Clost.* ufc/g	Lipoly. ufc/g	Proteoly. ufc/g
1	15.10^8	$3.0.10^5$	$5.0.10^4$	110	6.70	4.10^6	1.10^6
2	2.10^7	$3.1.10^5$	$3.2.10^4$	110	680	2.10^6	2.10^5
3	$1.2.10^7$	$1.1.10^6$	$7.0.10^4$	110	370	1.10^6	3.10^6
4	3.10^8	$3.0.10^4$	$3.0.10^4$	140	520	7.10^6	1.10^6
5	9.10^7	$4.7.10^4$	$2.1.10^4$	45	670	1.10^5	2.10^6
6	2.10^7	$3.0.10^5$	$8.3.10^4$	140	207	1.10^6	1.10^6
7	3.510^7	$2.7.10^5$	$1.7.10^4$	145	211	2.10^6	8.10^5
8	$1.2.10^7$	$1.1.10^5$	3.210^4	110	750	3.10^6	2.10^6
9	9.210^7	$7.0.10^5$	$4.0.10^4$	140	547	6.10^6	7.10^6
10	11.10^8	$4.1.10^5$	$1.7.10^4$	110	320	1.10^6	2.10^5
11	$2.5.10^7$	$2.2.10^5$	$2.4.10^4$	145	432	1.10^6	3.10^6
12	$3.2.10^7$	4.210^5	$2.1.10^4$	110	322	3.10^6	3.10^6
Average	1.610^8	$3.6.10^5$	$3.7.10^4$	120	494	$2.6.10^6$	$2.0.10^6$

Table 5: Physico-chemical analysis during the fermentation.

Parameters	initial product (Start of fermentation)	Final product (Fermented)
pH	6,42	3,5
Dry matter (%)	48,72	45.32
Total nitrogen (%)	11.2	10.25
NNP (%)	4,25	6,31
Total Protein (%)	20,8	21.25
Ash (%)	7.10	8.28
Fat (%)	9,6	9,8
Sugar (%)	30	23.5
Potassium (mg)	312,78	311
Phosphate (mg)	360,012	365
Calcium (mg)	300,074	300
Magnesium (mg)	32,032	30
Iron (mg)	2,564	2.1
Zinc (mg)	1,12	2.1

Table 6: Amino acid composition of fermented products (mg / 100 g of protein).

Amino acid	Under stabilized fish product
Asp.	93,8 ± 0,8
Glu.	129,3 ± 0,4
OH-pro.	ND
Ser.	44,0 ± 0,3
Gly.	64,0 ± 0,7
His.	29,3 ± 0,4
Arg.	58,4 ± 0,9

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Thr.	46,1 ± 0,3
Ala.	61,8 ± 0,4
Pro.	ND
Tyr.	33,7 ± 0,1
Val.	49,5 ± 0,6
Met.	33,3 ± 0,3
Ile.	40,7 ± 0,5
Leu.	75,0 ± 0,9
Phe.	38,7 ± 0,3
Lys.	88,4 ± 1,1
Cys.	ND
Trp.	ND

ND: not determined

Table 7: Evolution of microbial populations in the product before and after fermentation

Microorganisms	fermentation beginning	fermented products
Acid bacteria	$3{,}2.10^{6}$	$4{,}8.10^{9}$
FMAT	$1{,}6.10^{8}$	$6{,}1.10^{5}$
total *coliforms*	$3{,}6.10^{5}$	0
fecal *coliforms*	$1{,}1.10^{3}$	0
Clostridium sulfite reducers	$8{,}5.10^{4}$	no colonies
yeasts	$1{,}7.10^{3}$	$1.6\ 10^{4}$
Molds	80	0

Table 8: Evolution of stem diameter and leaf area of maize cultivation during the growing season.

Duration (months)	1	2	3
witness:			
Diameter (Average cm)	2.25	3.69	5.35
leaf area (cm^2)	16.5	32.32	49.73
Test :			
Diameter (Cm)	4.35	6.23	8.35
leaf area (cm^2)	24.15	59.21	71.27

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Fig. 1: Crops But after 3 months of treatment
Witness: Racine untreated plants (root biomass 150g ± 2.29 g).
Essay: Root plants treated with the extract of Bio-stimulant-rooting (root biomass of 750 ± 3.57 g).

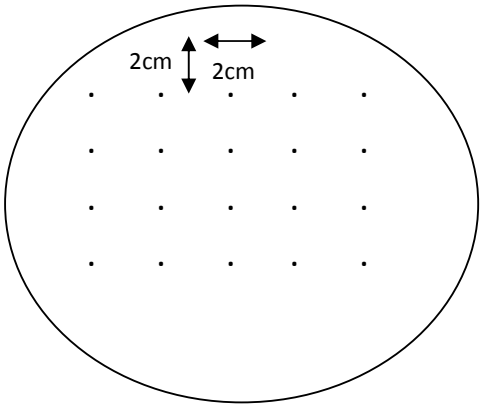

Fig. 1: Technical seeding pots

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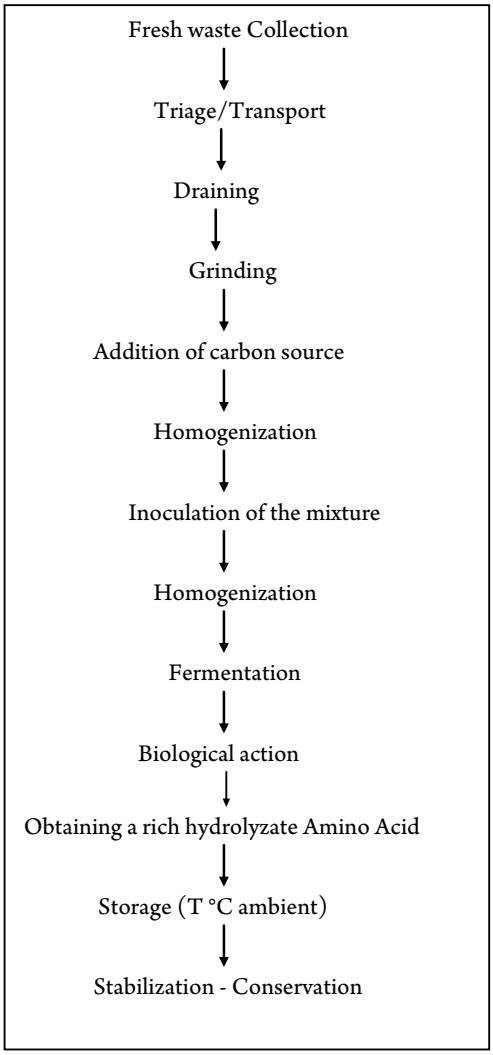

Fig. 2: General by-product of fish processing process

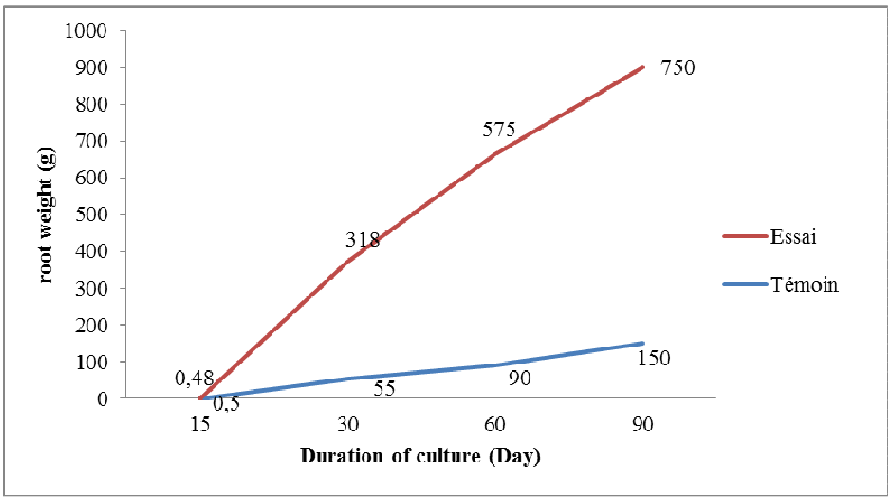

Fig. 3: Evolution of the weight of the corn root during its growth cycle.

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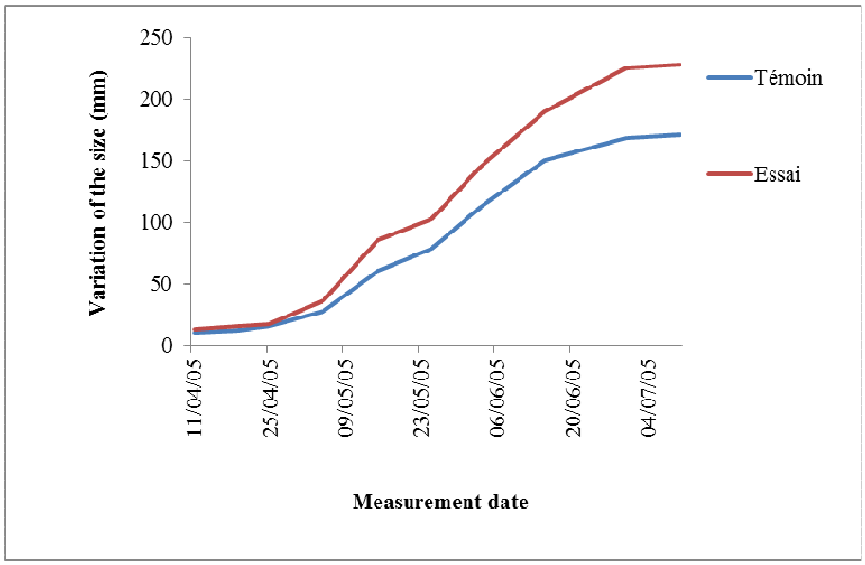

Fig. 4: Evolution of Maize size during its growth cycle.

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CONFLICTS OF INTEREST
"The authors declare no conflict of interest".