Available online at http://www.journalijdr.com

IJDR

ISSN: 2230-9926

International Journal of Development Research
Vol. 07, Issue, 11, pp.16773-16781, November, 2017

ORIGINAL RESEARCH ARTICLE | OPEN ACCESS

# BIOFERTILIZERS OBTAINED FROM FISH BY-PRODUCTS AIMED AT IMPROVING THE BIOLOGICAL FUNCTIONS OF SOILS AND PLANTS. APPLICATION TO CORN SILAGE

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**ARTICLE INFO**

*Article History:*
Received 19th August 2017
Received in revised form
25th September, 2017
Accepted 20th October, 2017
Published online 29th November, 2017

*Key Words:*

Biotransformation, Fermentation, Fishby-Products, Biofertilizers, Hydrolyzate, Biostimulants, Corn of Silage, Early Corn, Late Corn, Experiment in Field, Yield of Silage.

**ABSTRACT**

The national halieutic production reached in 2014 a quantity of 1353 780 tons. The industry of transformation and valuation of the products of the peach in Morocco occupies an important place in the national economy because it's treats about 70 % of the captures of the coast fishing and exports approximately 85 % of its production on hundred countries in the five continents. Now the pelagic fish industry generates a significant amount of waste up to 60%, are generally filed anarchically in the nature in the garbage dump and caused a negative impact on the environment and public health. Although, the analyses of these fish by-products showed a high load of pathogenic microorganisms, they also showed a wealth of organic material including proteins and minerals. These components and others are capitalizing to be used for agricultural or agro-food, hence the need for the establishment of an industrial process of their treatments and their valuations. That's why our laboratory, has made a biotechnological method based on the use of a fermentation leaven and carbon-rich source of carbohydrates, A hydrolyzate rich in amino acids and trace elements was obtained with excellent hygienic quality. It will be used as a fertilizer and soil improver. Its use will be as factors for fertilizer and soil amendment. We produced two biostimulants necessary for plant growth: Rooting-development, elongation-production. The application tests on these biofertilizers, on the culture of the corn of silage in the laboratory were performed. These tests have allowed obtaining promising results, so significant difference concerning the size of leaf surface and diameter of the different organs of the plant have been observed in comparison with the proof plants. Seen the importance of the corn of silage in the dairy production and considering the promising results that we have obtained in the laboratory tests, it was decided to extend these same tests on the cultivation corn in the field at breeders. Promising results are obtained in this experimentation, concretized by a highly significant overtaking in the development of the stems, leaves and ears of corn cultivation treated with biostimulants in comparison with those of untreated maize, confirm the results of the laboratory tests. The impact of the application of the biostimulants on the culture of corn was reflected, also on the ear yields, is an average number of 2,5 ears per plant with a weight 140g by ears for treated early corn against only 1.5 ears per plant and 60g/ears for the untreated early corn and 110g by ears for treated late corn against only 45/ears for the untreated late corn. Indeed, and as an indication, the analysis of the realizations of production showed one more time the highly significant effect of the application of the biostimulants. Indeed, the treated early corn gave a production of 119,04T/ha of silage almost 3 times more than the yield realized by the untreated early corn which did not exceed 43T/ha, also, the late treated corn gave a production of 111.6T/ha of silage is 3 times more than the yield realized by the untreated late corn with only 35.08T/ha of silage.

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**Citation: Hammoutou, S., Aziane, A., Aouane, E., Chaouch, A. and El yachioui, M., 2017.** "Biofertilizers obtained from fish by-products aimed at improving the biological functions of soils and plants. Application to corn silage.", *International Journal of Development Research*, 7, (11), 16773-16781.

## INTRODUCTION

The strong population growth in recent years has put pressure on arable land. The increase in population has also led to an increase in the demand for plant products that are useful to humans and animals. This strong pressure on the vegetation cover influences the capacity of the soil to produce the biomass necessary for the needs of a growing population.

Numerous long-term experiments carried out in the last decade of the twentieth century showed that national management of mineral fertilizers and organic amendments made it possible to increase crop yields and to sustain soil fertility sustainably. (Bado *et al.,* 1997; Bationo and Makwuney, 1999; Berger *et al.,* 1987;Pichotétal.1981; Piéri, 1989; Sédogo, 1981, 1993).

However, the demand for agricultural products and the pressures on resources and environmental impacts are increasing. In this context, new forms of agriculture are needed to continue producing more with less arable land, water, fertilizer or plant protection products. In recent years, various products and substances have been developed within the agricultural input market to improve the functioning of the soil, the plant or the interactions between soil and plant. This is a very broad category of products and substances that provide often innovative solutions in the field of fertilization and crop protection. The common feature of these solutions is that they rely on a mode of action through the stimulation of biological processes at the soil or plant level. The purpose of these products is to influence the ability of biological systems to adapt (for example: stimulation of the natural defenses of the plant or better absorption of nutrients, respectively).

They differ from agricultural inputs "conventional" because of their preventive nature, they do not directly address agronomic problems, but rather act as plant stimulants to promote better growth, improve the response to biotic or abiotic stresses. Bio-fertilizers or biostimulants for the plant are substances and materials which when applied to plants, seeds or growth substrates in a specific formulation have the ability to alter the physiological processes of plants in order to provide potential growth benefits, development and / or stress response. Anxious to contribute to the resolution of this scourge of a national character and which is harmful to the economy of the country especially to the small-scale breeders. Our laboratory has highlighted a biotechnological process for the processing and valorization of fish by-products (Patente, 1997) based on the use of an efficient fermentation leaven followed by a biological hydrolysis phase. A hydrolyzate rich in amino acids and trace elements was obtained with excellent hygienic quality. The hydrolyzate thus obtained was subsequently used as a source of amino acids in a formulation of fertilization products and soil amendment. Two bio-stimulants necessary for the growth of plants have been formulated by SINVA Morocco specializing in the manufacture of fertilizers and which exploits the patent of our invention.

A root-development biostimulant and biostimulant of elongation-production acting on the growth of the aerial part of the plants. The laboratory experiments on the effect of the above bio-stimulants on silage corn yielded highly significant results compared to the control that did not benefit from bio-stimulant inputs. (Hammoutou *and al.,* 2017), as shown in the following:

An increase in root biomass, crop size, and stem diameter of the leaf area during the vegetative cycle. With silage yields almost three times higher than the yield of untreated maize. The aim of this work is to confirm the reproducibility of the manufacturing process and to study the effects of bio-stimulants on cultivated plants, and saw the importance of silage corn in dairy production. It was decided to carry out large-scale trials on maize cultivation at the level of the field belonging to an agricultural cooperative.

## MATERIALS AND METHODS

### Consistency of experimentation

Experimentation in the field was installed at the cooperative "ALKHADRA" specialized in dairy farming, located 10 km from the town of Benslimane Region of Casablanca.

This cooperative, which brings together about 265 smallholders, has benefited from a Pillar II project on the development of dairy production under the Green Morocco Plan. However, the production of high-quality milk requires a balanced and constant feeding of cattle, the cultivation of corn silage is the basis of this diet, which is why our application on a large scale. The experiment was focused on a single variant aimed at studying the effect of the application of bio-stimulants on silage corn, knowing that the rest of the driving and maintenance operations will be the same for the treaty and the witness. Thus, a plot of 1000 $m^2$ irrigated with drip has been designated for the application of bio-stimulants, on the one hand on early spring corn. And on the other hand on late autumn corn. The Figure 1 Sketches the application test plot.

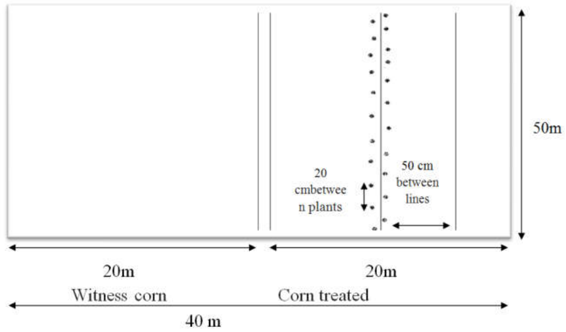


**Figure 1. Diagram of the test plot with 1000 $m^2$ for treatment with bio-stimulant and drip irrigation with staggered plantations**

### Installation of the culture

For the preparation of the seed bed, the cooperative tilled 25 to 30 cm with tooth tools followed by two passages crossed with the cover-crop to break the clods, aerate the soil of texture silty-sand and guarantee a thickest layer possible with the elimination of the weeds. Subsequently, about 25 mm of water was added to bring the soil back to its field capacity. The seeding was carried out manually for a density of 80.000plants per hectare in staggered along the irrigation pipes localized this conforms to the recommendations of the INRA Morocco (Anonymous, 1997).

### Fertilization

Taking into account the previous cultivation which is a fallow worked and on the basis of the results of soil analyzes, a fertilization program was carried out consisting of:

### Fertilizers (before seeding) at the rate of

- No intake of organic fertilizer;
- 2.5 Qx / ha of nitrogen in the form of ammonium sulphate 21%;

### Cover fertilizers

In the light of the results of analyzes, the inputs of cover fertilizers were reasoned:

**Inputs in macro-elements:** Ammo-nitrate 33.5%, 1.75 kg Soluble MAP(12-55,000) Solupotasse (50%) 1.25Kg.

**Micronutrient intake: (by foliar route)**sulphateof Zn (23%) 150 g/hl, Sulphate of Mn (32, 5%) 250 g/hl, Bore:Product to 15% of B 70 g/hl, Ironchelated EDDHA (6% Fe) 5kg/ha.

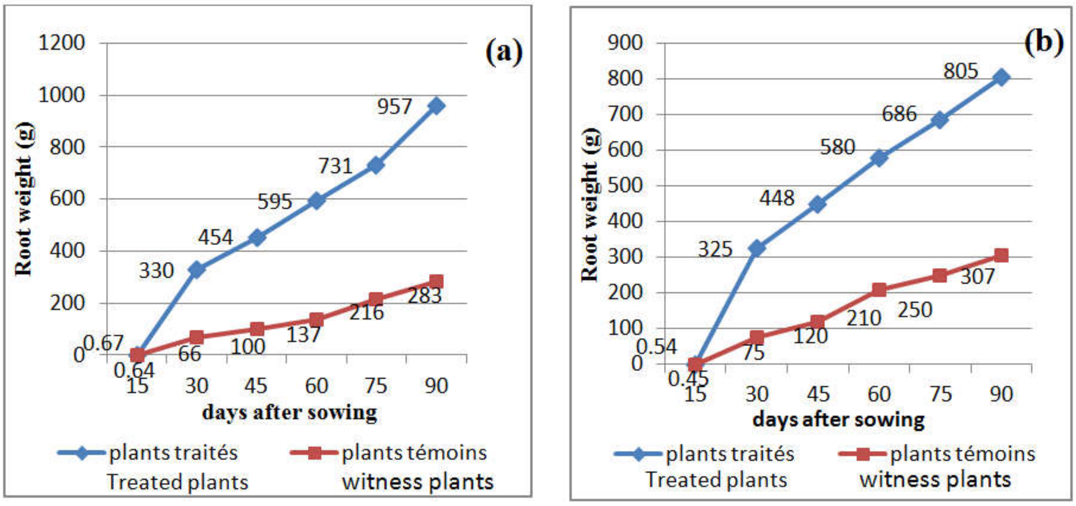


**Figure 2. Evolution of the weight of the maize root biomass during its vegetative cycle (a) Early cropping - (b) Late cropping)**

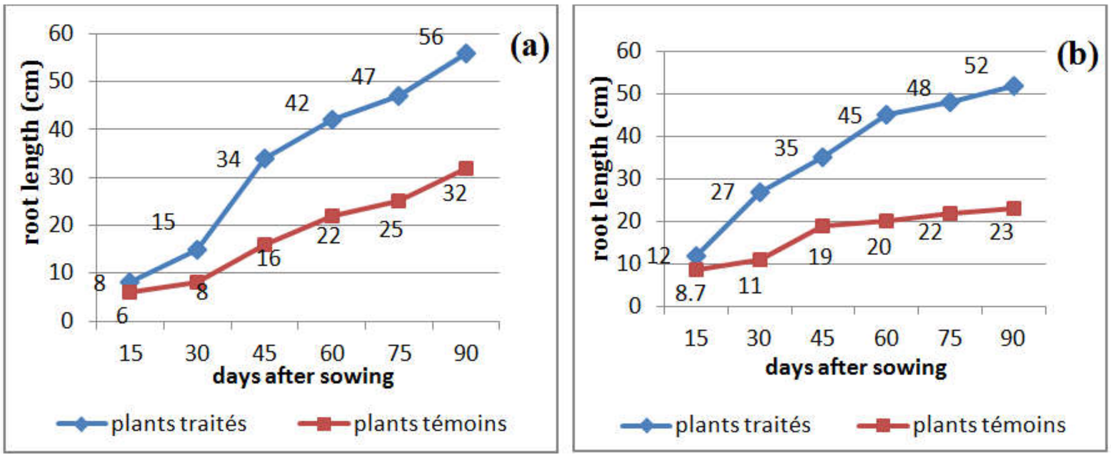


**Figure 3. Evolution of lengths of the longest roots of maize during its vegetative cycle (a) Early cropping - (b) Late cropping**

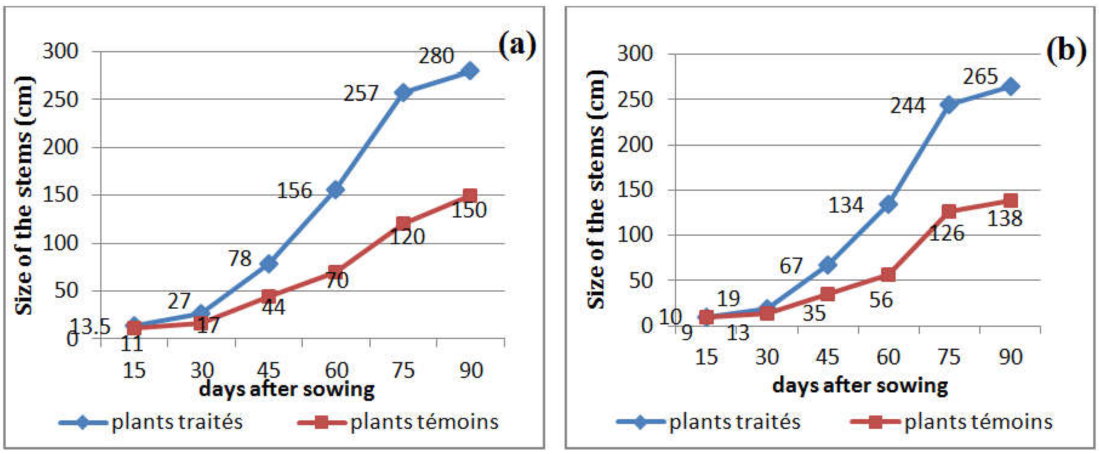


**Figure 4:Evolution of the stem size of maize during its vegetative cycle. (a) Early cropping - (b) Late cropping**

### Controls of badherbs

For weed control, which is essential for corn, it was done by manual intervention with female laborers and chemical using a post-emergence herbicide, 2.4-D(2.4-Acid - dichlorophenoxyacetic).

### Irrigation system

Before starting our application, an irrigation water analysis was used on this farm. The irrigation system used is drip at a flow rate of 0.16 liters /10min.

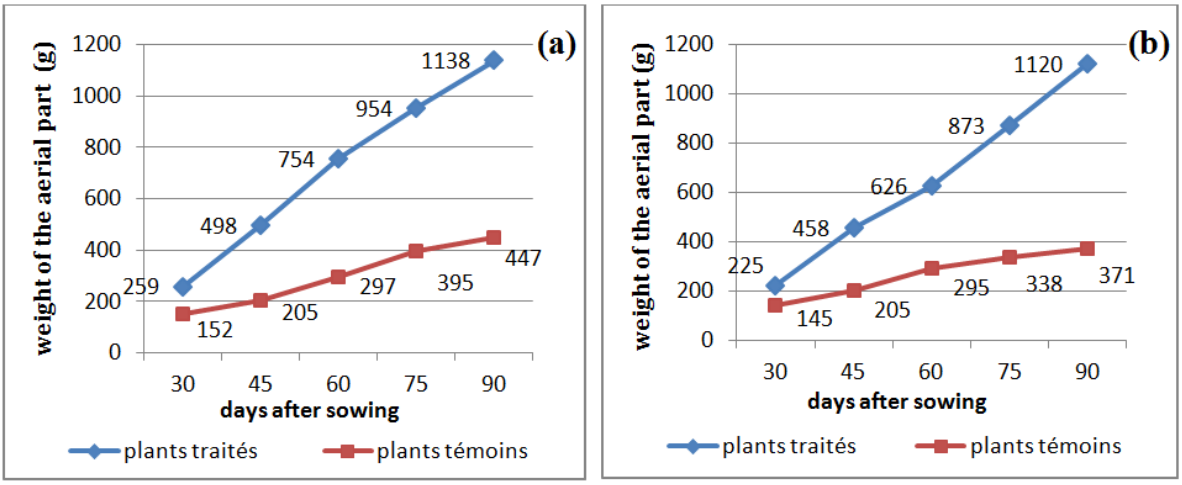


**Figure 5. Evolution of the weight of the aerial part of the maize during its vegetative cycle. (a) Early cropping - (b) Late cropping**

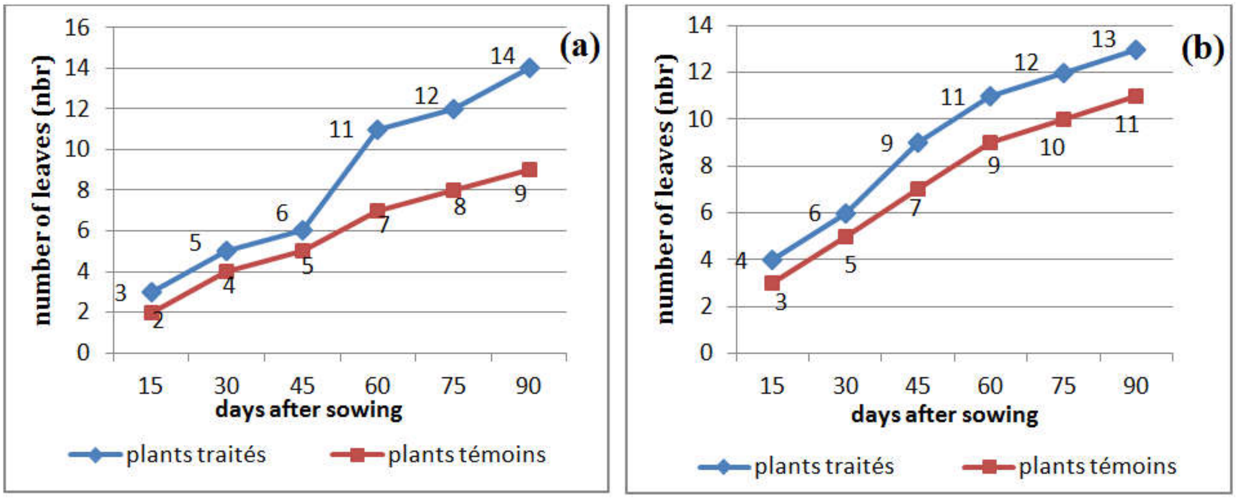


**Figure 6. The evolution of the number of leaves of the maize during its vegetative cycle. (a) Early cropping - (b) Late cropping**

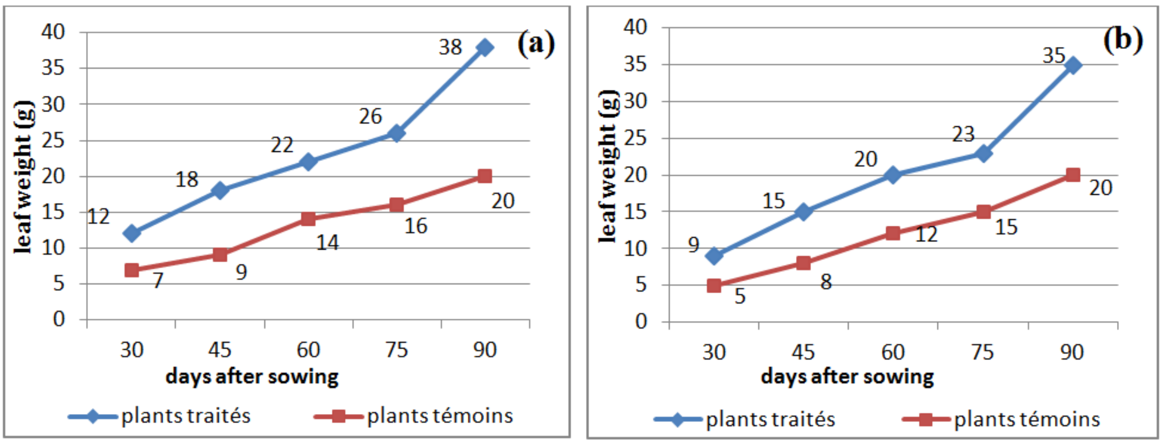


**Figure 7. The evolution of the leaf weight of maize during its vegetative cycle. (a) Early cropping - (b) Late cropping**

During the trial period, irrigation was carried out once every five days with doses varying according to the age of the crop, that to say a total intake of 4337 $m^3$/ ha.

**Bio-stimulants**

Bio-stimulants have been supplied by the company SINVA Maroc and are prepared from bio-based fish by-products adapted to the nutritional needs of plants.

Each formula differs depending on the purpose of the use of these bio-stimulants whose characteristics and composition are indicated on the packaging labels. In our tests two types of bio-stimulant were used.

### Bio-stimulant rooting

Its composition is shown in Table 1. It is applied by injection at a rate of 5 to 7 liters / hectare per application for vegetables and 7 to 9 liters / ha per application for Citrus and fruit trees.

**Table 1. Composition of Biostimulant Root**

| The composition | % (p/v) |
|---|---|
| Free amino acids | 8,0 |
| Total nitrogen | 17,5 |
| Uric nitrogen | 9,0 |
| Ammonia nitrogen | 3,0 |
| Phosphor($P_2O_5$) | 3,5 |
| Potassium oxide($K_2O$) | 3,0 |
| Polysaccharides | 3,0 |

Density = 1,2 pH=2,5

### Bio-stimulant acting on the growth of the aerial part

Its composition is shown in Table 2. Its application for the crops is carried out by foliar application at a rate of 150 to 200 cc / hl per application and in the irrigation water at the rate of 4 to 5 liters / ha per application.

**Table 2. Composition of Biostimulant acting on the growth of the aerial part**

| The composition | % (p/v) |
|---|---|
| Total amino acids | 30.00 |
| Free amino acids | 2.00 |
| Total nitrogen | 16.8 |
| Uric nitrogen | 7.0 |
| Ammonia nitrogen | 2.0 |
| Potassium oxide($K_2O$) | 3,0 |

Density = 1,2 pH=2,5 à 4

### Seeds

Application tests have been carried out on the cultivation of the maize variety "Colonia", it is a variety:

- Single Hybrid
- Toothed
- Mixed
- Forage seed drills(30% MS) 1850, recognized by these strengths, which are:
- Productivity and consistency
- Mixed grain forage
- Adaptation to any type of soil
- Excellent ratio whole plant on ears
- Very good performanceUFL /ha
- Very good resistance to lodging
- Good behavior against foliar diseases(Anonymous, 2007a)

### Taking action

Monitoring and evaluation of the effect of bio-stimulants along the culture cycle was carried out on measurements taken every day at a rate of 3 samples per dose. The parameters taken into account for the roots are the weight and length of the roots, for the aerial part the size and weight of the stems, the diameter, number, length and weight of the leaves, while for the fruiting stage, account of the weight of the ears and their number per plant.

## RESULTS AND DISCUSSION

### Application of root biostimulant

Marketed under the name **Mastro-R** and containing free amino acids to ensure a rapid and balanced nutritional intake, since the presence of polysaccharides, phosphorus and other essential trace elements in combination with amino acids makes it possible to have a system to increase the size and length of the primary and secondary roots, to stimulate the physiological functions of plants during periods of high activity (bud break, pollination, flowering ...).

### Effect on root biomass

From the 3-leaf stage after 10 days of cultivation of corn seeds, the bio-stimulant inputs were started. The first application of bio-stimulant Rooting was carried out with the doses indicated on the packaging of the liquid product marketed, at the rate of 5 L / ha via the irrigation system drop by drop every 15 days. The first sampling was carried out after 15 days of cultivation; the roots removed are washed, weighed and photographed. The results obtained (Figure2) show that the weight of the root biomass passes from 0.67 g to 957 g for early spring culture and 0.54 g to 805 g for late autumn culture against the untreated control which passes for the early maize from 0.64 to 283g and from 0.45 to 307g for late maize. There is also a significant difference in the number of rootlets that is higher in treated maize. Similarly, the comparison between the length of the longest roots of the treated corn and the untreated corn shows a remarkable difference between the two. Indeed, the follow-up of the evolution of the length of the longest root revealed for the early culture the evolution of the longest roots passes respectively from 8 to 56 cm against the control which passes from a value of 6 at 32 cm, whereas for late growing, it increases from 12 to 52 cm against the control which passes from 8.7 to 23 cm (Figure3). Also, the length of the smallest roots of the treated maize and the untreated root lengths showed that the shortest roots of the shortest roots decreased from 0.54 to 21.6 cm, respectively, from 0.45 at 11.4 cm, while for the late growing, it passes from 0.53 to 18 cm against the control which passes from 0.15 to 9 cm. In conclusion, the bio-stimulant root system has enabled the crop to have a rapid and balanced nutritional intake, since the presence of polysaccharides, phosphorus and other essential trace elements in combination with amino acids allowed to have a well-developed root system, increase the size and length of the primary and secondary roots, stimulate the physiological functions of plants during periods of high activity. In addition, the effect of application of the bio-stimulant on the root part made it possible to detect a development and a growth of the root biomass in comparison with the control maize which was not treated with this bio-stimulant. These findings are consistent with previous work on bio-stimulants, which states that root perception of a nutrient concentration in the environment results in the induction of signals leading to local changes in root and leaf architecture in order to optimize the collection of nutrients. These localized responses can result in increases in root growth and root diameter, and increases in the number of secondary roots. (Forde and Lorenzo, 2001, Mugnai *et al.,* 2008, Laëtitia, 2012).

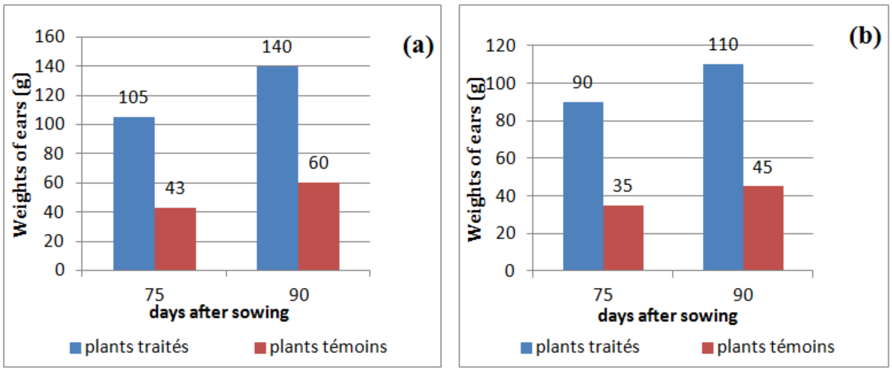


**Figure 8. The evolution of the weight of ears of corn during its vegetative cycle. (a) Early cropping - (b) Late cropping**

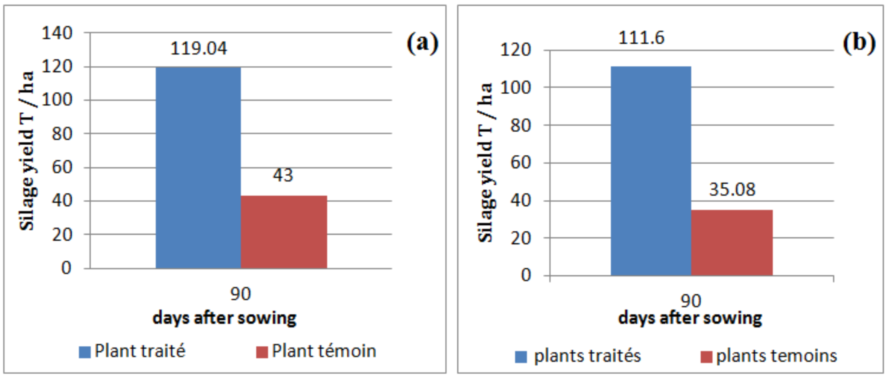


**Figure 9. Corn silageyield.(a) Early cropping - (b) Late cropping**

**Photo 1. Corn cultures after 3 months of treatment: great difference in growth and development of treated maize and control**

**Photo 2. Maize crops after 3 months of treatment: Treated: stem size of plants treated with Bio-stimulant-root extract can reach up to 2.85m in height**


**Photo 3. The overall appearance of treated corn and control corn after 90 days of culture**


**Photo 4. Early Corn Cultures, after 3 months of biostimulant application: plant biomass is highly important in treated maize than control**

These modifications occur not only in the number of secondary roots but also in the number of absorbent hairs. The root surface in contact with the medium is thus greatly increased for the treated plants. Our results are consistent with previous work, notably those of Schmidt *et al.,* 2007; Eyheraguibel *et al.,* 2008; Mora *et al.,* 2010; Laëtitia, 2012. As a result, this root biomass of treated corn would automatically allow the latter to better take advantage of the availability of water and mineral elements in the soil, which will translate into good development and productivity in terms of both quantity and quality. This root development also ensures a good anchoring of plants on the ground thus limiting the phenomenon of lodging.

## Effect of the application of bio-stimulant on the growth of the aerial part of maize

Marketed under the name ALFAMINE, it is a liquid product rich in free amino acids that can be applied at any time of the vegetative cycle. The application of the bio-stimulant was carried out by the foliar route and in the irrigation water. It can be applied during the vegetative cycle. In order to demonstrate the effect of the bio-stimulant on the aerial part of the maize crop, the evolution of the size and diameter of the stem was monitored, the number and weight of the leaves and as well as the distance between the nodes along the evolution of photosynthesis and the accumulation of soluble sugar during the vegetative cycle of the crop. The results obtained are recorded in the following:

### Effect on the development and growth of maize plants

The comparison between the development of the treated maize (early and late crop) and the untreated maize plants shows that the development of maize treated by the bio-stimulant root, elongation and production in root and foliar application exceeds that achieved by the plants that have not benefited from bio-stimulant. Indeed, this situation is declined as follows: The evolution of the size of the stems obtained clearly shows that the size increased significantly for the two trials, in fact, it passes respectively from 13.5 to 280 cm for early cultivation of the spring and 10 to 265 cm for late autumn cultivation against the untreated control for early maize 11-150 cm and 9-138 cm for late maize, respectively (Figure 4). Photos 1 and 2 illustrate the results on the field of cultivation. It should also be pointed out that the change in the diameter of the stems obtained clearly shows that it has increased significantly for both trials, in fact it passes from 0.6 to 3.5 cm for early culture and 0.3 to 3.2 cm for late growing against the untreated control which passes respectively for the early maize of 0.55 to 2 cm and of 0.4 to 1.85 cm for the late maize. This result coincides with earlier authors' work, emphasizing that there is an important relationship between the plant's nitrogen content, water intake and plant growth, the nitrogen content of the plant increases the diameter profile of the plant during the vegetative cycle (Roussos *et al.,* 2009; Spinelli *et al.,* 2010 ; Laëtitia, 2012). The evolution of the weight of the aerial part obtained clearly shows that it has increased significantly for the two tests, in fact it passes respectively from 259 g after 30 days of culture to 1138 g after 90 days of cultivation for Maize and 225 g after 30 days of cultivation at 1120 g after 90 days of cultivation for late maize against the untreated control which passes respectively for the early maize of 152 g after 30 days of culture at 560 g after 90 days of culture and of 145 g after 30 days of culture at 371 g after 90 days of cultivation for late maize. (Figure 5)

Moreover, the evolution of the mean space between the nodes of the aerial part obtained clearly shows that it increased significantly for both tests, in fact it passes respectively 9 cm after 30 days of culture at 20 cm after 90 days of culture for early corn and 5 cm after 30 days of culture at 17 cm after 90 days of culture for Late corn against the untreated control which passes respectively for the 5 cm early corn after 30 days of culture at 12 cm after 90 daysof culture and 4 cm after 30 days of culture at 10 cm after 90 days of culture for late maize.

### Effect of biostimulant on leaf number and growth

The comparison between the number and the growth of the leaves of the treated maize (early and late crop) and the untreated one shows that the maize treated by the bio-stimulants in root and foliar application showed a superiority compared to those not treated. This is illustrated by the following: The evolution of the number of leaves obtained clearly shows that it has increased significantly for both trials, in fact, it passes from 3 to 14 leaves for early culture (spring) and 4 to 13 (late) fall against the untreated control which passes for early 2- to 9-leaf maize and 3 to 11 for late maize respectively (Figure 6).

The evolution of the length of the largest leaves obtained clearly shows that it increased significantly for the two tests, in fact it passes respectively from 35 cm after 30 days of culture to 122 cm after 90 days of culture for early Maize and 30 cm after 30 days of culture at 99 cm after 90 days of cultivation for late Maize against the untreated control which passes respectively for the 20 cm early corn after 30 days of culture at 77 cm after 90 days of culture and of 18 cm after 30 days of cultivation at 75 cm after 90 days of culture for the late maize.

As for the weight of the leaves, the results obtained clearly show that it has increased significantly for the two tests, in fact it passes respectively from 12 g after 30 days of culture to 38 g after 90 days of corn cultivation and 9 g after 30 days of culture at 35 g after 90 days of late maize culture against the untreated control which passes respectively for the 7 g early corn after 30 days of culture at 20 g after 90 days of culture and 5 g after 30 days of culture at 20 g after 90 days of culture for the late maize (Figure 7).

**Effect of bio-stimulant application on Silage Corn Production**

**Effect on the weight of the Ears**

The comparison between the weight of corn on the treated corn (early and late) and the untreated corn (Figure 8 and Photo 3) shows that the average weight of corn cobs treated by the Bio-stimulants Rooting, elongation and production in root and foliar application is greater than twice the weight recorded in the ears of the control maize crop, with a value of 140 g for the early treatment, compared with only 60 g for the early control and 110 g for the treaty late to 45 g for the late control. As for assessing the effect of bio-stimulant application on yield, which is the main objective expected by livestock producers, it was approached through two indicators. This is corn yield and silage production.

**Effect of biostimulant on corn cob yield**

The comparison between yields of corn cobs (early and late) that benefited from bio-stimulant inputs and that grown without the use of bio-stimulants showed that the treated corn yielded corn 28T / ha compared to 7.2T / ha for early control and 22T / ha for late growing compared to 5.4T / ha for late control. The calculation is carried out on the basis of the following data:

- Density of planting: 80.000 plants /ha ;
- Average number of ears per plant: 2.5 for the treaty (early and late) and 1,5or the witness;
- Weight of ears:
- Early Corn: 140g
- Late corn:110g
- Early Witness: 60g
- Late Witness: 45g

**Effect of biostimulant on silage yield**

The harvest was carried out at the milky stage of the seeds and at a total dry matter content of 30 to 35%. The harvesting operation consists of a forage harvester that cuts and grinds the entire maize plants and throws it into a cart drawn by a tractor that accompanies the forage harvester to pick up the crop. The results of biomass yields of silage maize (Figure 9 and Photo 4) between early and late maize crops that benefited from bio-stimulant inputs compared to the non-bio-stimulant control that treated early maize yielded a silage of 119.04T / ha compared with 43T / ha for the early control and 111.6T / ha for late growing against 35.08T / ha whereas for the late control.

Nationally according to Ministry of Agriculture data (Anonymous, 2007), yield varies by location and year. In favorable areas, the average yield is 100-140 tons per hectare for corn silage and 60-80 t per hectare for seed corn. The moisture content of the wet grain is 35-45% and the water content of the Silage Ears is 40-45%. Laabioui, (2006) pointed out that for corn and sunflower crops a significant increase in corn and sunflower grain yields was observed at the plots as amended by the abattoir waste coproduct compared to those modified by chemical fertilizer and the other amended by manure. For example, for maize, yield was 762 seeds per ears for an amendment by this co-product (7T / Ha), compared to 462 seeds per ears for control and 682 seeds per ears for chemical treatment.

**Conclusion**

The results obtained during this experiment showed a significant evolution whether for early or late maize compared to the control and at all levels:

- Root biomass
- Aerial part of the plant (stem, leaves)
- Yield in ears of corn
- Silage performance

We can conclude that bio-fertilizers have positive effects on plant growth in all organs. It also increases the photosynthesis, lengthens the vegetative cycle, promotes pollination, improves the size and early maturity of the fruit and improves the overall condition of the plant, stimulates and increases the resistance of the plant under abiotic and abiotic stress conditions. The numerous parameters of stimulated growths specify the action of the biostimulants on the precocity of the development. Moreover, we have found that the supply of biostimulant as a liquid nutrient solution decreases the amount of water consumed to produce the same amount of dry matter and increases the total consumption of macro and microelements. This increase is significant for nitrogen, copper and zinc. This proves that the co-product obtained by the biotransformation of fish by-products has a high fertilizing capacity and can be used as an alternative or complementary to the chemical amendment.

Our results are consistent with those published in the INRA Morocco research work, underlining that a corn plant under conditions suitable to its water and nutrient needs can produce seeds an average of 150 to 330 g / ear (Anonymous, 1993). It also stimulates metabolism and improves the overall condition of the plant. The beneficial effect of the application of bio-stimulants can be explained by the direct action of bio-stimulant components on plant nutrition by acting either on the plant or on the soil. In contrast to untreated maize, there was a phenomenon of reduction of the filling of the ears with weakening of the stems and of the whole plant. This situation can be explained by the reduction of the active leaf area leading to a limitation of photosynthesis. This increase in aerial biomass is accompanied by an increase in stomata opening and chlorophyll content of the leaves (Russell *&al.,* 2006).

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