Chapter 16

Chitin/Chitosan's Bio-Fertilizer: Usage in Vegetative Growth of Wheat and Potato Crops

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Additional information is available at the end of the chapter

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Abstract

This chapter consists of valuing the chitosan to create bio-fertilizers as fertilizers without going through the composting process because of their richness in the nutrient base elements of plants: nitrogen and phosphorus. Physicochemical analyses of the chitosan focused on pH, dry matter, organic matter, nitrogen, phosphorus and potassium as well as IR and XRD. The samples thus prepared were monitored for 15 days. PH, temperature and conductivity were monitored daily. According to the physicochemical analyses of waste (nitrogen, phosphorus and potassium) and the nutritional needs of our selected crop (soft wheat, Arrehane variety which are 90-90-50 U/ha), several doses are then determined for the purpose of the optimal formula after their application on the crop. An application of bio-fertilizer on the potato was also undertaken. Follow-ups were carried out during this study, such as the monitoring of the vegetative growth of wheat and the mineralization of the soil via its physicochemical analyses. The results show that our bio-fertilizer is rich in nitrogen with 4.98% and phosphorus with 1.42% and mineralizes quickly on the ground while leaving the soft wheat to absorb its nutrients effectively and improving its growth properties, then giving good yields.

Keywords: chitosan, vegetative growth, wheat, potato, crops, bio-fertilizer

1. Introduction

The consumption is always higher and more diverse, which leads to a growing production of wastes in quality and quantity. This growth causes huge danger on the environment and hence on the human health [1]. So many organic wastes are generated then constantly to the world by the domestic and the halieutic industry [2]. The sector of fishing is a part of the strategic sectors in the world. It plays an important role in the global economy [3, 4]. The development of this sector is related to environmental issues, in particular to waste management. Indeed, the quantities of the halieutic waste are considered at several thousand, tons of waste a year as researched by Chabbar [5] and Afilal et al. [6].

These are randomly put in the existing systems of evacuation causing big problems [7]. One of these problems is the negative effect on the environment and human health El Moutawakila [8] and Benzakour et al. [9]. However, several studies have been interested in the evaluation and in the treatment of this waste. Some of them had studied the evaluation of their potential polluting [10].

Thus, the process of biotransformation for this type of waste seems to be the most suitable to resorb these problems. It corresponds to the elaboration of beneficial products, of natural origin, usable as bio-fertilizing for grounds [4] in substitution of artificial fertilizers. Moreover, the excessive application of artificial fertilizers for one of the most important agricultural processes in the world, the volatilization of ammonia in the air, the pollution of water resources causing their eutrophication, the degradation of the ground by their pollution attack of the cultures by phytopathogenic diseases [11–13].

This biotransformation consists then of the spreading of bio-fertilizers, which are in the form of dried, crushed and spread waste in agricultural plots.

Indeed, direct spreading is the simplest method of valuation requiring the least investment; it provides nutrients, improves soil quality with water retention and stimulates microbial activity. However, composting does not seem to be favorized in the logistics (time and local manufacturing, odors, etc.), due to the limited supply of carbon materials and environmental constraints [14] such as the attraction of insects and plants, pests and the risk of weeds in crops [15].

In this context, the bio-fertilizing potential of seafood, that is, chitin/chitosan, is explored for wheat and potato crop. They represent in fact a rich organic source for organic farming. The objective of this chapter is to valorize seafood waste, considering it as a source of bio-fertilizers and not only as a source of pollution, thanks to a simple and inexpensive process by spreading them directly in the agricultural environment. Their valorization always allows the protection of the environment and the acquisition of a new economic source.

2. Study of the use of raw chitin-chitosan as a bio-fertilizer

2.1. Inconveniences of nitrogenous chemical fertilization

In agriculture, chemical fertilizers are administered to increase crop yield. They provide the nutrients that plants need to grow. There are several chemical forms of nitrogen fertilizers in the market and the distinction between them is made possible through the various conventional chemical tests. The choice of one form over another is often difficult because of the contradictions in the published results on the composition of measures of agronomic efficiency [16].

The trio nitrogen, phosphate and potassium (NPK) is the basis of all these products, and they are also responsible for massive soil pollution but are especially the major cause of pollution of groundwater, the main reservoirs of drinking water. If they change their environment and make the water unsafe, there are certain dangers listed in **Table 1** [17].

The dangers of nitrogen fertilizers (nitrates)
- When they are not consumed by plants, they easily seep into the soil and progressively reach the groundwater.
- They are soluble in water.
- Before the 1950s, the nitrate content per liter of water did not exceed 1 mg. Nowadays, it easily exceeds 50 mg/l.
- Contribute to the phenomena of eutrophication [18].
- They are degraded by a bacterium and turn into nitrites that can poison the blood by oxidizing hemoglobin. The fluid then poorly fixes oxygen and causes respiratory problems.

Table 1. Some dangers of chemical fertilizers.

2.2. Contribution of waste to soils

The return to the ground of waste has been practiced by man since always. There are two reasons to explain this ancestral practice: first, the fertilizing value of this waste and then the capacity of the soil to purify the effluents, in particular, liquids, which makes it possible to protect the deep and surface waters against all risk of pollution. Strengthening regulations on the protection of the environment should make it possible to sustain this agricultural recycling while preserving the quality of the receiving soils, crops and water [13]. Waste spread in agricultural fields comes primarily from agriculture itself or from industries directly linked to it. Depending on their chemical composition, waste can be brought to the soil to provide fertilizer amounts equivalent to mineral fertilization (**Table 2**).

Some residual products are brought to the soil as an amendment. An amendment is a contribution to soils for the main purpose of improving their physico-chemical and biological properties. The organic matter content of a soil is one of the key elements leading to a stable structure and helping to limit the risk of soil erosion, especially in soils with a silty texture [19, 20]. However, the use of waste as fertilizer or amendment can only be accepted if their negative environmental impacts are minimal compared to their positive effects. With increasing public awareness of the need to preserve soil quality as well, the risks associated with land application now include not only the plant and water aspects but also those of soil and even air.

2.3. Physico-chemical preparation and characterization of the raw chitin

The waste of shrimp (DC) was naturally collected and dried. These samples are crushed and sieved in 2 mm (**Figure 1**).

Type of waste	% Material dries	N (g/kg)	P (g/kg)	K (g/kg)
Solid sewage sludge	55.2	24.8	8.3	1.7
Foams of sugar refinery	29.0	7.4	4.0	0.9
Liquid manure of poultry	13.3	10.2	1.8	5.4
Fertilizer of cattle	28.4	6.2	1.4	5.9
Fertilizer of sheep (mutton)	29.3	8.6	1.8	11.0
Compost of fertilizer of bovine	35.2	7.6	1.3	6.1

Table 2. Average contents of fertilizing elements in waste spread in agriculture [18].

Figure 1. Shrimp waste after drying and grinding "DC".

The analyses focused on pH, nitrogen (N), phosphorus (P), potassium (K) and organic matter [17]. The characterization of raw chitin is performed using IR spectroscopy and X-ray diffraction (XRD).

2.4. Results and discussions

The results of the physico-chemical analyses made on the chitin [17] are summarized in **Table 3**.

These measures are in accordance with the International Standards for AFNOR, the NF U44-051 standard approved in 2006 for fertilizers of plant and/or animal origin, the amendment of which allows the soil to be maintained or stockpiled of its existing organic material as well as the improvement of the physical, chemical and biological properties of the soil [21, 22]. This standard stipulates the following contents:

N < 3% on MB, P_2O_5 < 3% on MB, K_2O < 3% on MB and N + P_2O_5 + K_2O < 7% on MB.

MS ≥ 30% MB and MO ≥ 20% MB depending on the type designation and C/N > 8.

MB: raw material; MS: dry matter; MO: organic matter.

According to the results obtained, our bio-fertilizer ratifies almost all the values of the AFNOR standard quoted above.

Indeed, the sum of the percentages on N, P and K is less than 7%, it is 6.45%. The percentages of phosphorus and potassium are 3% lower except that in nitrogen, the MO content is

Waste/parameter	pH	DM (%)	OM (%)	C (%)	N (%)	P (%)	K (%)	C/N
SW	8.55	26.13	56	28	4.98	1.42	0.05	5.62

Table 3. Results of physico-chemical analyses of the raw chitin.

greater than 20%. The MS is close to the norm. On the other hand, the C/N ratio is lower than the norm.

According to the analyses, shrimp waste is rich in nitrogen. The composition of its waste (carapaces, viscera, small portions of flesh attached and associated water) is characterized by its high nitrogen content, which is granted with several previous works [15, 23, 24].

This richness is due to the high crude protein content; a factor of 6.25 was used to convert total nitrogen into protein. The percentage of protein in our sample is therefore 31.12%; this value is approximate to those of other research: protein contents of 52 [25], 44.12 ± 0.79 [26] and 47, 43 and 47.75% [23].

Ravichandran et al. also reported [27] that the percentage of crude protein in the dry matter of raw chitin is 24.03%. Similarly, a percentage close to these values of 29.3% was found by Prameela et al. in 2010 [28]. Also, Khan and Nowsad [29], in 2012, found high percentages ranging from 40 to 50% of proteins in shrimp shells.

These results showed that shrimp waste is high in phosphorus, which is in agreement with many authors who have found that this content in the head and shell is, respectively, 0.017 and 0.029% [23]. This phosphorus richness is attributed to its contribution to the formation of crustaces structures and their strengthening when phosphorus is combined with calcium.

The shrimp waste had an alkaline pH (8.55); this value was found by Mohan et al. which is 8.10 ± 0.10 [28, 30, 31].

2.4.1. Infrared analysis

Infrared spectrum of raw chitin.

Figure 2 shows the Fourier transform infrared (FTIR) absorption spectra of crude chitin. The positions of the various bands observed and their attributions are summarized in **Table 4**. The spectrum shows broad vibration bands located at 3100–3500 cm^{-1} corresponding to the ─NH and ─OH elongation vibrations including the hydrogen bonds. Two absorption bands appear in the two 1557 and 1652 cm^{-1} spectra due to the ─CO─NH_2 elongation vibrations. These bands of amide I and amide II of chitin are more easily identifiable in the case of chitin. The spectrum of chitin shows bands in the region 500–900 cm^{-1} called the region sensitive to the structure.

The infrared spectrum is used to illustrate the presence of nitrogen in the fertilizer matrix. It is almost similar to the spectrum of chitin found by the author Boukhlifi et al. [30, 31].

2.4.2. DRX analysis

Figure 3 represents the diffractogram DRX of the waste of shrimps.

The observation of the X-ray diffraction spectra of shrimp waste shows the presence of two intense peaks at $2\theta = 9.9°$ and at $2\theta = 19.9°$; these results are identical to those obtained by Ahlafi et al. [31], with pure chitin; these researchers showed that *α*-chitin has two peaks of diffraction, $2\theta = 9.3°$ and $2\theta = 19.4°$. Other authors, Liu S at al. 2012, [32] showed that chitin

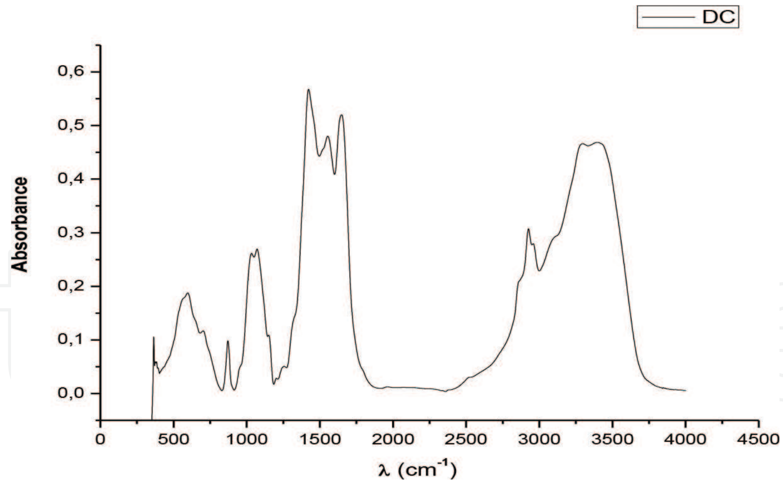

Figure 2. Infrared specter of the waste of shrimp.

Band cm^{-1}	Nature of vibration or rotation
500–900	Region sensitive to the structure
1028	Vibration of strain of the C─O─C of the cycle glucosidique
1089	Vibration of strain of ─OH
895 and 1153	Liaison glucosidique β(1→4)
1254	Vibration of distortion of O─H
1423	Symmetric distortion of CH_3 and CH_2
1557	Amide Il
1652	Amide I
523, 741 and 1652	Identifies the chitin
2880 and 2923	Strain of ─CH and CH_2
3100–3500	Strain NH and ─OH, including the binding of hydrogens

Table 4. Characteristic vibration bands of chitin and chitosan.

has a strong reflection at 2θ = [9–10°] and at 2θ = [20–21°] and a minor reflection at 2θ = 26.4°; we can conclude therefore that the characteristic peaks of chitin exist in the analyzed waste and appear in our spectrum; we can still see in the spectrum that there is an intense peak at 2θ = 30°, which is due to the calcite present in shrimp shells and in the chemical fertilizers used. The diffractogram also shows that the mineral part of our sample contains a mixture of two varieties: calcium carbonate and $CaCO_3$/calcite, syn [17, 19].

2.5. Choice of the culture of execice

This chapter consists of adding raw chitin to a bio-fertilizer while applying it to soft wheat (*Triticum aestivum*), Arrehane variety and potatoes. A comparison with two witnesses was made, the first is the commercial chemical fertilizer (ammonitrate, 21%) the most consumed

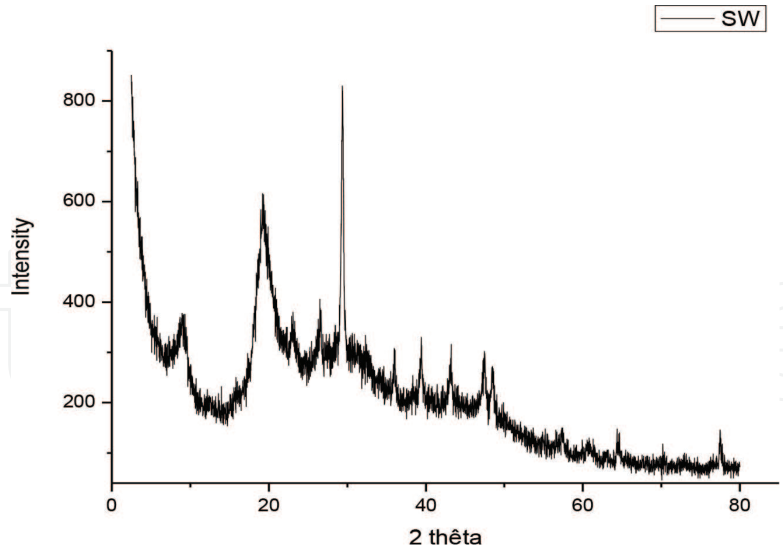

Figure 3. Diffractogram DRX of the waste of shrimp.

in Morocco with 27% [16] and the second is no fertilizer. This is to improve the biofertility of the soil, which is a fundamental value for organic pioneers.

The analyses of the waste used and the soil samples of the test were carried out at the National Institute of Agronomic Research to determine the fertilizing power of the waste and to follow the evolution of the mineralization of the soil.

2.6. Application on soft wheat

2.6.1. Cultivation of soft wheat

The determination of organic inputs for crude chitin in kg/t in terms of N, P and K was made on the basis of the results of physicochemical analyses of waste and the requirements of common wheat in these same elements are 49.8 kg/t [17].

Soft wheat, variety Arrehane, was sown at a rate of 15 seeds per pot with a surface area of 0.07 m^2, simulating a seeding rate of 214 seeds per square meter. The sowing was in November 2014, the growth period started and ended at the end of June and the harvest took place in July 2015. The temperature and lighting are natural ambient. Watering was done as needed with well water.

The test is done in pots with one-third of sand and two-third of soil (**Figure 4**). The organic input doses (in g/pot) (**Table 5**) were calculated based on the shrimp waste content in N, P and K elements as well as the requirements for soft wheat in these elements, which are 90–90–50 kg/ha [20]. Four treatments were predetermined on the basis of nitrogen fertilizer content (N); the tested treatments are 100, 150, 200 and 300%. This method is similar to that of Yadav et al. [21]. A chemical fertilizer (EC) treatment is also applied to wheat with the same treatment and an absolute control where the soil has received no fertilizer.

The monitoring of the crop is carried out there after measuring the growth parameters of the wheat until it reaches maturity. Each sample then consists of the ears of wheat harvested in

Figure 4. Some steps of substrate preparation and sowing.

Treatment waste (%)	SW (g/pot)	EC (g/pot)
100	14	3
150	21	4.5
200	28	6
300	42	9

Table 5. Organic and chemical input rates for wheat cultivation.

all pots of the test. These ears were then shredded with the electric thresher and the recovered kernels were weighed for determination of estimated crop yield.

2.6.2. Soil tests

Soil samples of 0–20 cm were taken three times during the wheat growth cycle at the time of spawning, tillering and at maturity in all pots of the experimental set using a stainless steel tool. The samples were put in the laboratory for measuring the fresh weight, then are dried in an oven for 48 h at a temperature of 60°C for carrying out analyses of the elements P, K, organic matter and pH and conductivity measurement according to the internal protocols of INRA.

a Content in potassium

The extraction of the potassium in the ground was made by the addition of extract of the ground (acetate of ammonia). In every sample to extract all the elements of the ground by means of Wheaton-Omnispense more and make shake flasks in an agitator goes and comes hanging (AGITELEC) 30 min. After these stages, samples are filtered. The reading of the content of filtrates in potassium is made on the photometer for flame:

$$K_2O\ (ppm) = ppm \times 10 \times 1.2 \tag{1}$$

b Phosphorus content

Phosphorus analysis was performed using the 0.5 M sodium bicarbonate ($NaHCO_3$) extraction solution at pH = 8.5. The same filtration process is thus carried out, extract was taken and put in Erlenmeyer flasks and then sulfuric acid (5 N) was added to acidify the solution. The staining solution was then added. This supplemented with distilled water; the solutions of the calibration range were also prepared in order to plot the calibration curve to deduce the phosphorus concentrations. The intensity of the blue color of the solutions is read at 820 nm after 15–30 min.

The calculation formula is as follows:

$$P\,(\text{ppm; soil}) = \text{reading}\,(\text{ppm}) \times 20 \tag{2}$$

c Soil content of organic matter

To know the amount of organic matter present in the soil, 1 N of potassium dichromate, sulfuric acid (H_2SO_4), distilled water and concentrated phosphoric acid (H_3PO_4) were used.

In the last step, a few drops of the indicator (diphenyl amine) were added. After homogenization, the excess of $K_2Cr_2O_7$ with 1 N $FeSO_4$ was titrated to bright green and the volume of $FeSO_4$ was recorded under the same conditions as a blank solution without a soil sample.

The calculation is done by the following formula:

$$\text{OOC}\,\% = \frac{\left(\left(V(\text{white}) - V(\acute{e}ch.titr\,\acute{e})\right)\right) \times 2 \times 0.3 \times 0.5}{\text{weight of the ground}} \tag{3}$$

$$\text{TOC}\,\% = 1334 \times \text{OOC}\% \tag{4}$$

$$\%\text{M.O} = 1724 \times \text{TOC} = 2.3 \times \text{OOC} \tag{5}$$

d pH analysis

A quantity of soil is suspended in a double volume of distilled water (5 g of soil/10 ml of water). The mixture is stirred with a glass rod. The mixture was allowed to stand for 30 min, stirred 5 or 6 times during this period, and then the pH was measured.

2.6.3 Results of growth monitoring of common wheat and comparison of root volume and weight

At the end of the test, roots and residues were extracted by a stream of water that caused the soil to settle downward and the exclusion of floating roots upward. The roots of the wheat were then removed from the soil, washed and cleared of soil debris and then oven-dried at a temperature of 60°C for 48 h.

The growth of common wheat was followed throughout the season by length measurement. At harvest, several parameters were measured, namely:

- final length of plants (cm)
- weight of grains/pot (g)
- grain yield estimate (ton)/ha
- root volume and weight

a Wheat growth for each dose

Figure 5 shows the growth of wheat, based on the doses of treatments that are 100, 150, 200 and 300%. In general, the average growth data showed that wheat grew well in all the increasing doses provided by the biological treatment (raw chitin) ending up to almost 99 cm in length (150% treatment) and exceeds the growth of wheat receiving the chemical fertilizer at all these doses and the witness which did not undergo any contribution. For the 100% dose, the wheat amended by the raw chitin exceeds in length that is amended by the chemical fertilizer with 6.33 cm and the control with 13.53 cm, which is the same for the other doses of 150, 200 and 300%, successively, with 10.27, 5.37 and 1.1 cm for the chemical fertilizer and with 20.73, 19.5 and 12.16 cm for the witness.

These results do not corroborate those found in the study of the effect of raw chitin on radish cultivation [15] where the length of radishes fertilized by mineral fertilizers exceeds that of radishes amended by shrimp residues with 1.1 cm. On the other hand, they are corroborated with the work of Taiek et al. where they demonstrated that the fish waste allied to malting releases allowed for an optimal growth of barley and tomato, better even than the commercial fertilizers [5].

b Comparison between doses

The growth of wheat fertilized by doses 150 and 200% of the gross chitin exceeds that of the other two doses; the wheat fertilized by the 300% dose is good growth but the latter has declined toward the end of the growth cycle (from April 29 to May 06, 2015). This is due to the excess of nitrogen in the soil and therefore is absorbed by the wheat plants, which causes a delay in the maturity phase [33] unlike, the others; it is concluded that the 300% dose is limited to growth. From there, we can recommend that the following doses corresponding to treatments 150 and 200% of our bio-fertilizer are equivalent successively to 3 and 4 t of N/ha which is equivalent to 135 and 180 U N/ha (**Figure 6**).

The wheat grows very closely with regard to all the doses of the chemical fertilizers brought. At the end of the growth cycle, the wheat amended with the 200% dose outgrows the other wheat plants. That is the dose that corresponds to 180 U of nitrogen per hectare, and this is the recommended dose per hectare by the INRA in Morocco which varies between 160 and 200 U/ha [34].

c Yield results of soft wheat

At maturity, soft wheat was harvested manually from ground level, and the harvested biomass was weighed later. The grains were separated from the straw with a drummer, and the grain yield was recorded after weighing.

Applying our only bio-fertilizers significantly increased wheat yields compared to chemically fertilized wheat; (**Figure 6**) it is a maximum yield of 30 q/ha for bio-fertilizers against 16.18 q/ha for commercial fertilizer treatment 200% corresponding to the contribution of 180 U of

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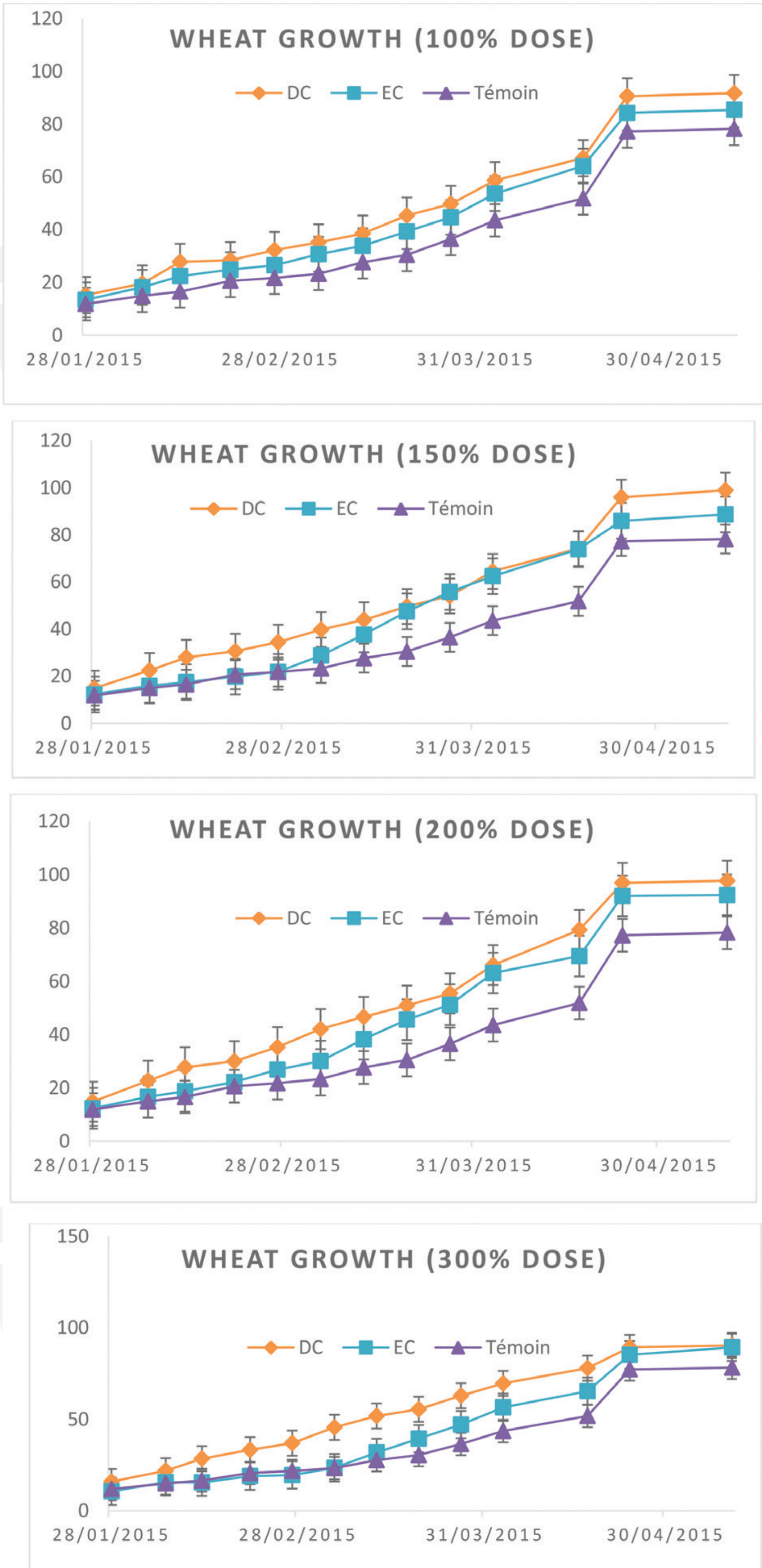
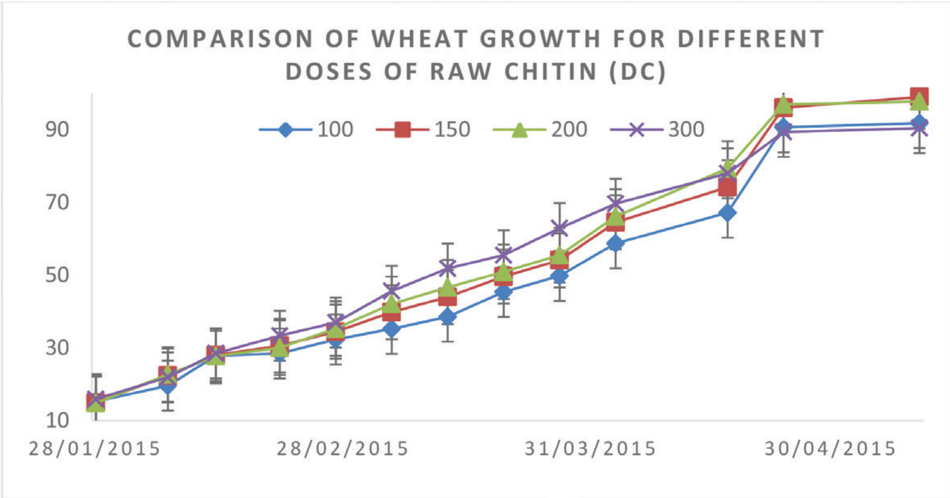
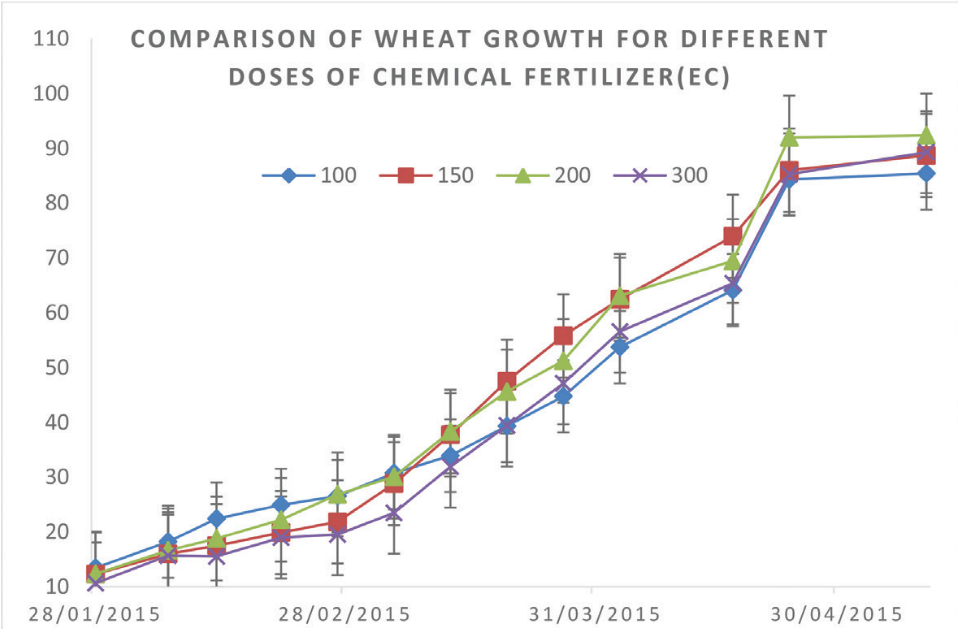


Figure 5. Comparison between bio-fertilizer and chemical fertilizer in terms of wheat growth for each dose: (a) 100, (b) 150, (c) 200, and (d) 300%.



(a)



(b)

Figure 6. Comparison of growth of the wheat for all the doses of the used fertilizers (a) DC and (b) EC.

nitrogen per hectare, which approves a correlation with growth well developed in wheat fertilized by the gross chitin with the same treatment above. According to the Ministry of Agriculture at the national level, the average yield of common wheat has increased, between the periods 2000–2007 and 2008 and 2015, from 14.3 to 19.2 q/ha, a value lower than that found for our organic fertilizers [35].

In addition, application of marine residues has been shown to have positive effects on crop yields. Abdel-Mawgoud [36] has shown that a foliar application of chitosan on strawberry plants helps to increase the height of the plants, the number of leaves and even the yield of strawberries. Abdel-Mawgoud et al. [37] also noted an increase in fall triticale yield following the early spring application of mussel residues. Also, Karine has shown that treatment with pure marine residues, dried shrimp waste, has produced the best radish yields

[14]. In addition, the authors state that composting reduces the availability of nitrogen from marine residues. Indeed, some of the nitrogen is lost through volatilization during the composting process. After fermentation, the decomposition of the fresh residues generates toxic compounds (volatile fatty acids, lactic and acetic acids, etc.). Some studies have highlighted another benefit that corresponds to the safener effect of marine residues or marine residue extracts on various diseases. In 2006, ADAS [38] stated that the addition of crustacean residues stimulates soil microbial activity, which can promote competition between soil microorganisms at the expense of pathogenic microorganisms.

d Results of comparison between volume and weight racinaires

The role of roots is very important for the absorption and the transport of the water and the mineral elements toward the air parts of plants [39]. Their development intervenes in the evolution of the properties of the ground and more particularly its structure and its content in organic matter [40]. The roots of the wheat fertilized by the bio-fertilizer exceed in weight and volume, though some wheat is fertilized chemically; (**Figures 7**, **8** and **9**) our results confirm the results

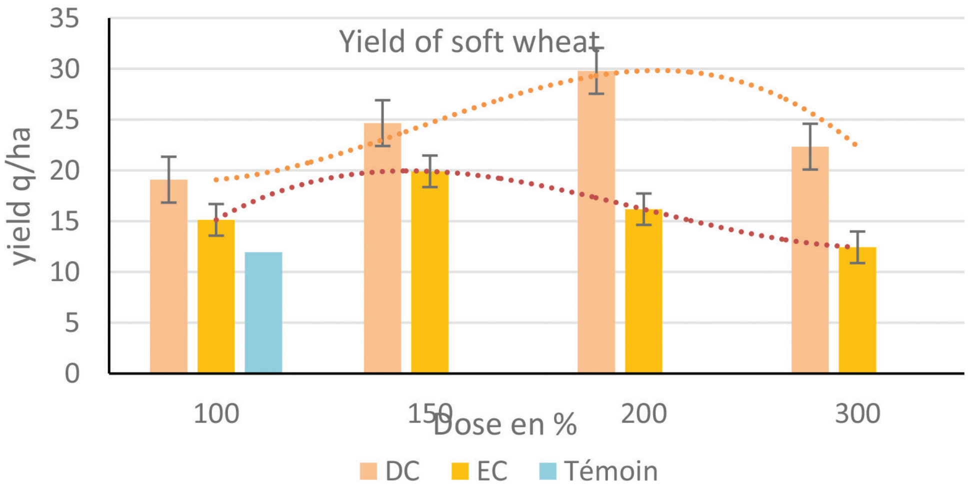


Figure 7. Yield on the common wheat.

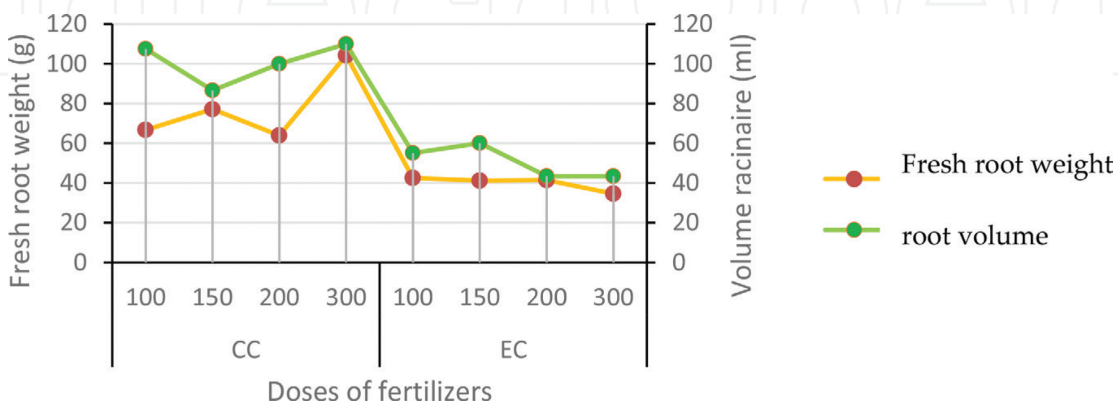


Figure 8. Comparison between the bio-fertilizer and the fertilizing in terms of volume and weight racinaires some common wheat.

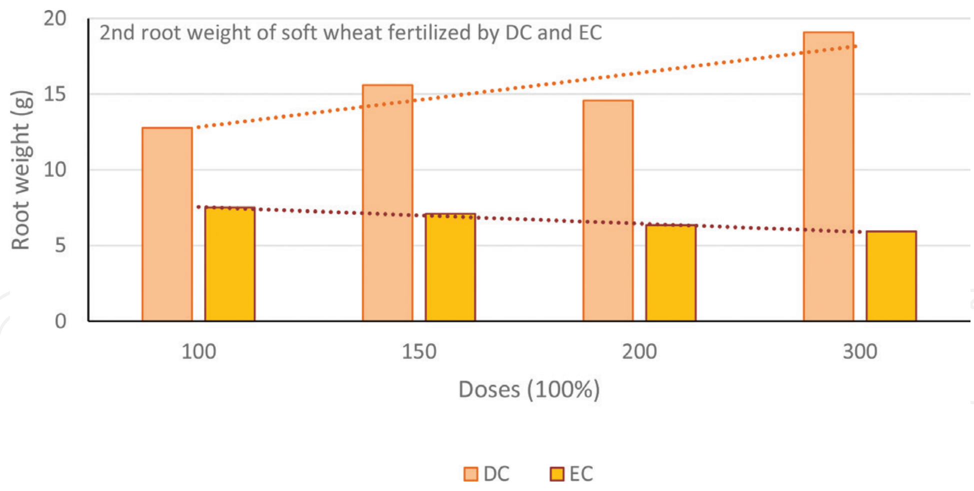


Figure 9. Secondary root weight of soft wheat.

of Karine [14] where the diameter and the biomass of roots for radish fertilized by the waste of shrimps are superior with regard to those of radish which is fertilized by the artificial fertilizer.

e Evolution of soil mineralization

Table 6 represents the mineralization of total organic carbon (percentage) during the growth phases of soft wheat.

The mineralization of organic matter is a process of degradation. Its main consequences are the decrease of the organic matter content in a soil and the selective disappearance of certain compounds [41, 42]. The decomposition of organic matter is expressed in terms of mineralized carbon and types of molecules present in the soil over time [43].

However, the effects of bio-fertilizers on biological activity and mineralization of nitrogen in the soil depend in particular on their nitrogen concentration and their C/N ratio [44], which further confirms the results found for our bio-fertilizer from shrimp waste where the nitrogen content is 4.98%. The bio-fertilizers with lower C/N ratios are mineralized rapidly in the soil, releasing significant amounts of nitrogen absorbed by subsequent crops [14, 45–47] that also

Dose	DC			EC		
	After lifting-tallage	Tillering-montaison	Bolting-heading	After lifting-tallage	Tillering-montaison	Bolting-heading
100	23.84	96.28	−48.14	17.91	98.95	−66.86
150	28.20	113.66	−53.49	24.88	98.95	−48.14
200	31.22	108.31	−45.47	27.62	100.29	−50.81
300	35.17	100.29	−56.16	18.49	82.91	−34.77
Témoin	40.87	73.55	−34.77	40.78	73.55	−34.77

Table 6. Total organic carbon mineralization (%).

states, for this study, the C/N ratio which is 5.62. Besides the carbon decomposition rate has peaked at 31.22 and 35.17% at post emergence-tillering phase and 113.66 and 108.31% at tillering-stem extension phase for bio-fertilizer doses used, respectively, at about 200 and 300% which explains the high content of the soil in this element following its total decomposition. This increase is also explained by the decrease in the mineralization potential, which corroborates the work of Martel et al. [48] and Karine [14]. The regular supply of residues or manure can increase the total organic C of the soil to a higher equilibrium level, related to the balance between C inputs and decomposition processes [48]. And it is the same—the decomposition of the carbon element in the soil for the wheat fertilized by the chemical fertilizer is maximum for the doses of 150 and 200%, successively at 24.88 and 27.62% (after the emergence-tillering phase) and 98.95 and 100.29% (tillering-stem extension) for doses of successively 100 and 200%.

Subsequent to the cycle, we find that 53.49 and 56.16% of the carbon element was mineralized during the stem extension-heading period; those are maximum values for the bio-fertilizer doses of 150 and 300%, respectively. For the chemical fertilizer, 66.86 and 50.81% values of the mineralized carbon (maximum values) are determined for doses of 150 and 300% too. This is where the microbial communities convert the carbon element provided by the bio-fertilizer and the chemical fertilizer into stable C in the soil, as studied by Kallenbach et al. [49].

2.7. Application of the bio-fertilizer on potatoes

2.7.1. Preparation of the ground

The slightly acidic pH (pH = 5.5 or 6) of the soil can give good yield. Excess alkalinity of the soil can cause development of the common tuberous gall.

The high salinity of the soil can block the absorption of water by the root system, so to complete in combination with these conditions, the soil preparation is an essential step for the best fertilizer application. The preparation techniques consist of ensuring good contact between the tubers and the soil because the development of the root system will usually be delayed if the soil is poorly prepared. The soil should be prepared to a depth of at least 25–30 cm; such a loose layer promotes aeration of the soil, ensures good root development and facilitates ridging.

A good seedbed can be done as follows:

- medium plowing (25–30 cm) with plow;
- spreading of dry waste and phospho-potassium fertilizers with doses well respected according to the needs of the potato/ha;
- a good preparation of the first 10 cm of the soil allows a good cover of the plant.

Fertilization remains one of the most important factors for good potato production. For a production of 25 tons/ha (tubers + haul), the requirements of the amount of essential elements for the plant include 160 kg (N)/ha, 45 kg (P_2O_5)/ha, 275 kg (K_2O)/ha, 50 kg (MgO)/ha and 70 kg (CaO)/ha.

The potato is very demanding in organic manure, the needs are of the order of 30 T/ha and this dose can be doubled in soil low in organic matter. The maximum nitrogen absorption takes place at the time of 50–80 days after planting. Nitrogen can be applied in the form of sulfate of ammonia; the phosphorus is hardly absorbed by the plant for it must be applied before planting and in the most assimilable form. The doses should be divided into three periods; emergence, first hump and second hump.

The recommended doses are only average and must be adapted according to the richness of the soil. A preliminary soil analysis is necessary to evaluate the level of soil fertility. Nitrogen should be located at the ridges while avoiding direct contact between the plant and the fertilizer.

2.7.2. Plantation of the potato

The tubers of the potato are classified according to the following sizes: from 28 to 35 mm, from 35 to 45 mm, from 45 to 55 mm and calibers greater than 55 mm. Planting density of 15 to 20 stems/m^2 gives good land use; pre-germinated 35–55 mm of plant produces approximately 5–6 main stems, usually 4 plants/m^2 with a distance of 70 cm between lines and 30 cm between plants; the seed requirements/ha equals about 2000 up to 2500 kg. Planting tubers at a uniform depth that depends on the type of soil, climatic conditions, and the physiological age of the plants gives a homogeneous culture.

The choice of planting at 5–6 cm of depth is applied in moist, heavy soil as the mother tubers may run out before the germs reach the soil surface, but for a textured soil, where a slight or a risk of drying out is to be feared, the planting is often done at a depth of about 10 cm.

2.7.3. Irrigation of the potato

The organization of activated irrigation promotes and ensures the mechanisms of transport of mineral elements, synthetic products, transpiration and thermal regulation at the leaf level because the potato is very sensitive to both deficit of water and excess water; it requires water which is evaluated between 400 and 600 mm according to the climatic conditions, the type of soil and the length of the cycle. According to the following frequency:

- During germination, the amount of water needed is low.
- For 60 days up to 90 days after planting; irrigation should be carried out at very short intervals (6 or 7 days) in light soil and for 12 or 15 days in heavy soil up to 10–20 days before harvest.

The quality of irrigation is measured by the rate of salts which must be less than 4 g.

2.7.4. Earthing-up

The operation of earthing-up consists of returning the earth to drive the mound; the purpose of this operation is of cover the superficial roots of the plant, the nitrate and potassium fertilizers applied during the culture, and prevent the culture of the moth (decreased the attack of insects).

The first planting is done 2 to 3 weeks after the late emergence of the plant, the length of the plant must be at least 10cm above the ground. It is necessary to stay up during harrowing not to affect the system racinaire and the recently formed tuber, this operation is important because it consists in taking all the weed. The yellowing of the lower leaves, the drying of stalks and the firmness of the skin of tubers are factors of the harvest after 3–4.5 months. The lifting must be made in dry weather and tubers should not be left exposed too much to the sun to avoid the development of the black spots and the attack by the moth.

2.7.5. Hilling

The humping operation is to bring the previously loosened earth to the ridge to form the hill; the purpose of this operation is to

cover the superficial roots of the plant, the nitrogenous and potassic fertilizers applied during the cultivation and prevent the cultivation of ringworm.

The first hilling is done 2–3 weeks after the lifting, after a hoeing operation, so as to cover at least 10 cm of soil, and then the operation is repeated every 2–3 weeks.

Care should be taken during hoeing not to touch the root system and newly formed tubers, this is important because it involves taking all the weeds.

Yellowing of the lower leaves, drying of the stems and firmness of the tuber skin are factors in the harvest after 3–4.5 months.

Tearing should be done in dry weather and care should be taken not to leave the tubers too exposed to the sun to avoid the development of black spots and ringworm attacks.

2.7.6. Application of bio-fertilizer: raw chitin

The application of raw chitin on the potato in this study is performed qualitatively in bags.

- initialization of the bottom of the bags by 2 cm sand sole to seeds of large pores to facilitate the permeability of excess water during irrigation;
- preparation of a mixture of sand-clay-soil (one-third of the sand + two-thirds of the clay soil);
- addition of a 5 cm layer of soil that we have prepared so as to not lose fertilizer doses by the permeability of water;
- tuber planting in another layer of soil mixed with 50% of the initially determined doses to a depth of 7 cm;
- monitoring of buddies by irrigation according to the needs of the water needed for the growth of the potato during planting and after so as to keep the soil moist;
- the soil after the first hilling must be mixed with one of the 25% of the doses;
- the same operation for what concerns the second hilling with the last 25% of the doses, after having the germs on the surface and the formation of the stems.

Time (J), after survey	Number of main stems (petioles)	Length of petiole max (cm)	Length of compound leaf max (cm)	Number of compound leaves	Length of secondary leaf (leaflet) max (cm)	Width of secondary leaf (leaflet) max (cm)	The length of the plant (cm)	Number of secondary petioles
9	3	5.5	9266	7666	4166	3233	9.5	
12	3	8	14.333	13.666	6566	5733	13.333	
16	3333	13.666	25.666	23	8	6266	23.5	
19	3333	19.666	28	24	9.5	6,4	31.666	
23	3333	28	29	27	10.433	9666	38	
26	3333	31.333	30	35.333	10.93	9,5	40.666	14
30	3333	33.833	30.5	32.333	10.933	9633	41.833	15
33	3333	35.666	30.666	33.666	11	9833	45.5	17
37	3333	37	31.5	35.333	11.133	9,9	46.666	19
40	3333	39.666	33	37.666	11.333	10	48	19

Table 7. Potato monitoring in soil contains 300% of N requirements in raw chitin.

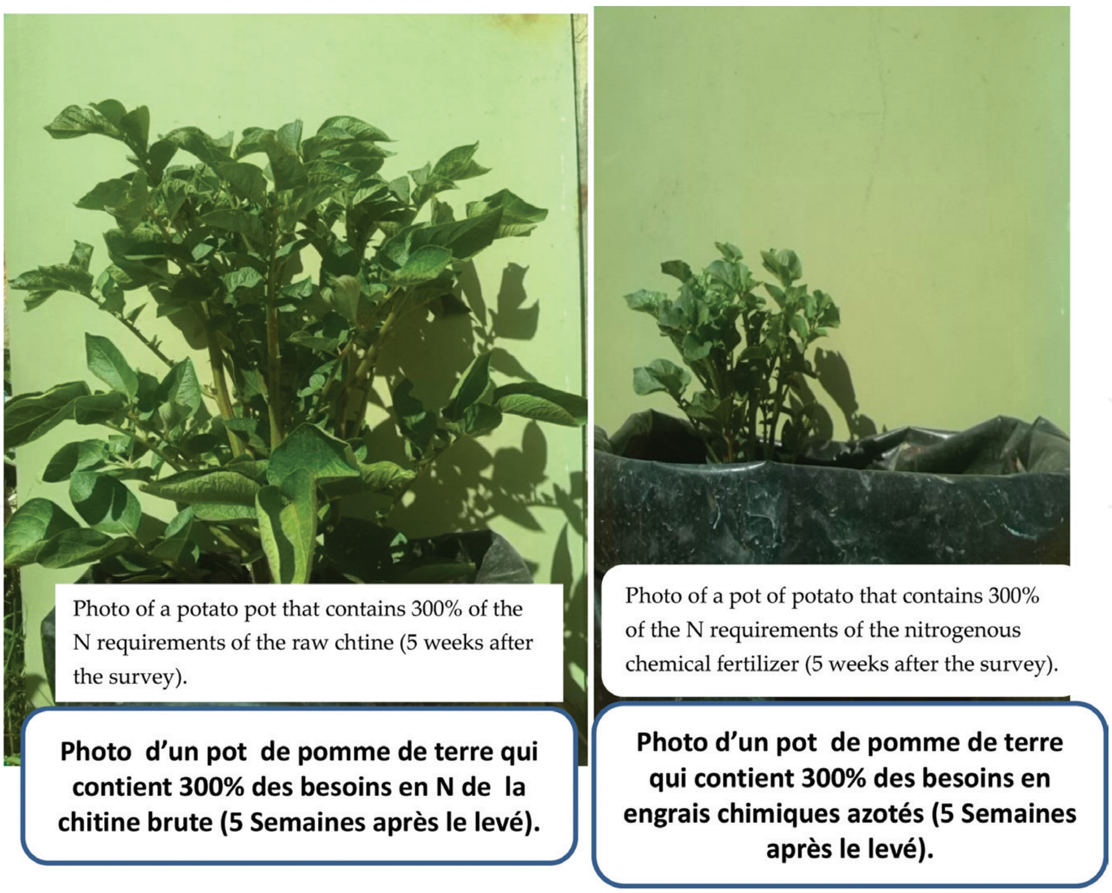


Figure 10. Growth of the potato according to the fertilizer used.

2.7.7. Study and follow-up of the factors indicating the good growth of the potato

The length of the plant, the length of the petioles, the length of the compound leaves and the length and width of the leaflets are all more important factors that promote photosynthesis that allows the transformation of mineral matter into plant tissue. They increase the volume of the flowering and improve its precocity, help to increase the weight of the seeds, lengthen the plant and increase the protein content of the seeds, so all these factors are important to obtain at the end of the harvest a good performance. Thus, in **Table 7**, the analyses and measurements performed on these growth factors are grouped together. We also give pictures (**Figure 10**) of some pots on which we made our measurements.

Figure 10 illustrates well that the potato in the case of the fertilization by the raw chitin is well pushed compared to the case of fertilization by the chemical fertilizer. This proves that there is a significant degradation of chitin which brings a significant amount of nitrogen to the plant.

3. Conclusion

As far as this study is concerned, the dehydrated marine residues, such as the shrimp waste, mainly raw chitin, represent an interesting source of nitrogen and carbon for the agricultural valorization. Consequently, their treatment produces wheat yields significantly higher than those fertilized with chemical fertilizers. The raw chitin is more effective than chemical fertilizer and more effective than the test.

So, we recommend the dose to bring from 200% corresponding to 180 U of nitrogen of our bio-fertilizer which is the raw chitin for the cultivation of soft wheat, variety Arrehane. The richness of shrimp was also proved by the quality and quantity of potatoes obtained after fertilization with raw chitin. The biological degradation of chitin and the important contribution of nitrogen have been proved by vegetative growth.

The use of bio-fertilizers from organic waste as chitin/chitosan remains a useful and sustainable practice. It is necessary for the reduction of these environmentally harmful wastes' rate to increase the agricultural yields and to have an effective maintenance of soil fertility.

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