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SCAN ME

Research Paper

EXTRACTION OF CHITOSAN FROM THE EXOSKELETONS OF TWO SPECIES OF CRABS (*Callinectes amnicola* AND *Cardisoma armatum*) AND EVALUATION OF ITS EFFECTIVENESS ON *IN VITRO* GERMINATION OF MAIZE (*Zea mays* L.) IN BENIN

Toussaint Mikpon[1,2], Nadège A. Agbodjato[1], Durand Dah-Nouvlessounon[1], Olarewadjou Amogou[1], Halfane Lehman[1], Christine N'tcha[1], Pacôme A. Noumavo[3], Sylvestre Assogba[1], Marcellin Allagbe[2], Hyacinthe Ahissou[4], Adolphe Adjanohoun[5] and Lamine Baba-Moussa[1]

[1] Laboratory of Biology and Molecular Typing in Microbiology; Department of Biochemistry and Cellular Biology / Faculty of Science and Technology (FAST) / University of Abomey-Calavi (UAC).
[2] South Agricultural Research Center, National Institute of Agricultural Research of Benin; BP 03 Attogon, Benin.
[3] Laboratory of Microbiology and Food Technologies, Department of Plant Biology, (FAST / UAC, 04 BP 1107 Cotonou, Benin.
[4] Laboratory of Biomembranes and Proteins, Institute of Applied Biology (ISBA), University of Abomey-Calavi (UAC).
[5] Agricultural Research Center of Agonkanmey, National Institute of Agricultural Research of Benin,
Benin.

Abstract

Agriculture has become a major issue in the face of an increasing population and growing food needs. The objective of the study was to produce chitosan from the exoskeletons of two crabs' species (*Callinectes amnicola* and *Cardisoma armatum*) for use in growing maize in Benin. For this purpose, the shells of each of the two species of crab collected were washed, dried and then ground. The two chitosans samples were obtained after demineralization in acidic medium, followed by deproteination in basic medium, then bleaching and hydrothermal chemical deacetylation in basic medium. The effects of the extracted chitosans were evaluated on the in vitro germination of corn seeds for seven (07) days in Petri dishes. The results obtained showed that the exoskeletons of these species are a good source of chitin with chitosan yields of 12.48% (*C. amnicola*) and 5.57% (*C. armatum*). After seven (7) days of germination, the results obtained show very good seed viability with a germination rate ranging from 94.44% to 97.22%. The longest seedling length was obtained by seeds treated with chitosan extracted

from *C. amnicola* at 0.5 g / l for an increase of 31.71%. The seeds treated with *C. armatum* at 0.5 g / l induced a good improvement in the vigor index, weight of roots, seedlings and germinated seed with respective increases of 20.57%, 42.70%, and 22%. The present study confirms that sources of chitosan from crustacean exoskeletons are available in Benin and show the potential of using this chitosan as a biostimulant to improve productivity and increase corn yield.
Key words: Crab exoskeletons, chitin, chitosan, maize cultivation, valuation, Benin.

INTRODUCTION

Improving soil fertility is one of the common strategies for increasing agricultural production. It therefore becomes important to develop different methods of improving soil fertility and biological control through the use of natural products and / or organisms, in order to reduce the effects of harmful organisms and improve agricultural production while protecting the environment. The appearance on the agricultural input market of various products and substances aimed at improving the functioning of the soil, the plant or the interactions between soil and plant through the stimulation of biological processes arouse the interest of actors in the agricultural world [1]. Among these substances is chitosan (a derivative of chitin extracted from the shells of crustaceans).

Chitosan is a natural biopolymer derived from chitin, a polysaccharide present in the exoskeleton of crustaceans and insects as well as the cell wall of fungi and certain algae [2]. Commercially, chitin is obtained from crustacean shells (shrimps, crabs and other molluscs) and its deacetylation makes it possible to obtain chitosan [3]. Several works refer to the stimulating effect of chitosan on crop growth due to the increased availability and absorption of nutrients and the process of photosynthesis through the accumulation of metabolites and the increase of pigments. foliar [4-7]. One of the sources of chitosan being crabs, a raw material available in Benin whose exoskeletons can be used for its production.

Indeed, Benin annually produces a large quantity of crabs. According to Rurangwa et al. [8], 70% of the total production of crabs from Benin is exported to Togo and Ghana. The lagoon systems of southern Benin contain a wide variety of crustaceans including *Cardisoma armatum* and *Callinectes amnicola* [9-11]. These species are among those subject to active fishing and commercial exploitation [12]. Despite the increasing human consumption of these products, the enormous quantities of waste generated are simply discharged into the marine environment or into public landfills, creating in the process serious pollution problems. The biodegradation of crustacean shells is very slow [13]. There is an urgent need to process and use shrimp and crab waste, which contains several bioactive compounds such as chitin, pigments, amino acids and fatty acids [14]. Based on the different production procedures for chitosan encountered in the literature [15-16] and adequate research, one of the solutions to the problems of its availability at the national level is its production from crab exoskeletons. It is therefore necessary for

agricultural research to position itself in the development of processes to make chitosan and bio-fertilizers based on microorganisms available to producers in the agricultural sector in order to improve agricultural productivity.

MATERIALS AND METHODS

Material

The exoskeletons of fresh water (*Callinectes amnicola*) and ground (*Cardisoma armatum*) crabs were used in this study to extract chitosan. Reagents such as hydrochloric acid (HCl), sodium hydroxide (NaOH), distilled water, acetone, ethanol etc., were used in the procedure of extracting chitosan from exoskeletons crabs. The corn seed used during the germination test was of the EVDT 97 STR W. It comes from the Center de Recherches Agricoles Sud (CRA-Sud) of INRAB. This variety shows good resistance to American rust, streak, helminthosporiosis, curvulariasis and drought [17].

Extraction of chitosans from exoskeletons of *C. armatum* and *C. amnicola*

Collection, drying and crushing of crab samples (pretreatment)

The samples of land crab (*Cardisoma armatum*) and freshwater crab (*Callinectes amnicola*) used were collected from anglers and crab sellers respectively in the municipalities of Adjohoun and Abomey-Calavi in Benin.

After collection, the shells were separated from the flesh and washed with tap water and distilled water, respectively, before being dried. To activate the drying process, the shells were sprayed with acetone and steamed for 24 hours at 50°C. Drying was continued in a Pasteur oven at the same temperature (50°C) for 10 days. After drying, all samples of each species were crushed and powdered using a Retsch type SM 2000/1430/Upm/Smfet.

Extraction of chitosans from the different powders of *C. amnicola* and *C. armatum*

The powders of the two species of crab were used for the extraction of chitosan according to the method of Oanh et al. [15]. For each species, the chitosan was obtained in four steps.

Step 1 - Demineralization (decalcification)

After the pretreatment, a mass of 500 g of dry powder per type of crab was thoroughly mixed in a 2-liter Erlenmeyer with a solution of hydrochloric acid (HCl, 2M). Throughout the experiment, the solid-liquid ratio is 1:10 w / v (dry shell weight / volume of HCl solution, 2M). A volume of 600 ml of the hydrochloric acid solution (HCl, 2M) was introduced into the Erlen, then placed under magnetic stirring overnight at a temperature of 50°C in order to solubilize the calcium carbonate and the chloride of calcium following the reaction below:

$$CaCO_3 + 2HCl \longrightarrow CaCl_2 + CO_2\uparrow + H_2O$$

After reaction, the contents of the Erlen were filtered through a filter paper placed over a Büchner funnel placed on a vacuum flask connected to a vacuum pump. Then, washing

was continued until neutral, in order to remove excess reagent. The powders obtained are dried in an oven at 50°C overnight.

Step 2 - Deproteination

Each of the dried powders was placed in a flask fitted with a condenser containing a solution of sodium hydroxide NaOH at 10% with the solid / liquid ratio 1:20 (w / v). The 300 ml mixture was brought to a temperature of 100 ° C in a heating mantle for 6 hours.

Vacuum filtration followed by washing with distilled water and then with acetone were necessary until neutral, in order to remove any proteins that were separated from the chitin. The recovered material was steamed at 50°C overnight.

Step 3 - Bleaching

This step consisted in removing the pigments from the shells of crabs which form complexes with chitin, such as β-carotene derivatives. In order to obtain almost white chitin, a preliminary extraction with an acetone / ethanol mixture was carried out with the proportions liquid: liquid 1: 1 (v / v), to which was added the dry matter obtained during the previous step , with a ratio of solid to solvent 1:10 (w/v) and left under magnetic stirring overnight. Then filtration was done, followed by washing with distilled water, then with ethanol and baking at 50°C overnight. The dry matter is again treated with an oxidizing agent which is hydrogen peroxide, at a concentration of 0.315% with a ratio of solid to solvent 1:10 (w / v), with magnetic stirring maintained for 30 min. It was filtered and washed with distilled water before being dried in an oven at 50°C overnight.

Step 4 - Synthesis of chitosan by deacetylation of chitin

This is the most delicate step which involves the substitution of a maximum and sufficient number of acetyl groups (CH3CO-), to result in chitosan. Deacetylation is usually carried out by treatment with concentrated sodium (NaOH) or potassium (KOH) hydroxide (40-50%) at a temperature ≥ 100°C. In our case, the experiments of this step were carried out with a NaOH concentration of 10M, at a temperature of 100°C for 180 minutes. The product was filtered and then washed with distilled water until neutral. To speed up the dehydration process, it was rinsed with ethanol before being placed in the oven overnight at 50°C.

Effect of Acetic Acid Concentration on Chitosan Solubility

The study of the effect of the concentration of acetic acid on the solubility of chitosan was carried out on solutions at concentrations of acetic acid ranging from 0.1 to 2.5M. The curves representing the solubility rate of chitosan based on *C. amnicola* and *C. armatum*, as a function of the concentrations of the acetic acid solutions were used to analyze the degree of solubility of the chitosan.

Evaluation of the *in vitro* effects of chitosans from *C. amnicola* and *C. armatum* on germination of maize

Experimental design

The effects of the extracted chitosans were evaluated on the germination of corn seeds according to different concentrations. The experimental setup used was a block of 05

treatments with three repetitions. The treatments were defined as follows: T1 = Controls (Seeds with sterile distilled water); T2 = CALI 0.05 (seeds soaked with chitosan extracted from *C. amnicola* with a concentration of 0.05 g / l); T3 = CALI 0.5 (seeds soaked with chitosan extracted from *C. amnicola* with a concentration of 0.5 g / l); T4 = CARDI 0.05 (seeds soaked with chitosan extracted from *C. armatum* at a concentration of 0.05 g / l); T5 = CARDI 0.05 (seeds soaked with chitosan extracted from *C. armatum* at a concentration of 0.05 g / l).

Preparation of the chitosan solution

For the preparation of the solution of the two types of chitosan based on *C. amnicola* and *C. armatum*, the concentrations used were respectively 0.5g / l and 0.05g / l per liter of distilled water.

Inoculation and germination

The corn seeds were soaked for 2 min in a 0.024% Sodium Hypochlorite solution then rinsed five (5) times with Sterile Distilled Water [18]. The seeds thus disinfected were soaked in the chitosan solutions prepared beforehand and contained in the Erlenmeyer flasks with stirring for 12 hours depending on the concentrations defined for each treatment. After 12 hours of soaking, 12 seeds were removed and placed equidistantly using sterile forceps in a sterile 11.8 cm^2 Petri dish, lined with sterile blotting paper beforehand soaked in 10 ml of sterile distilled water. The inoculated seeds were then covered with blotting paper soaked in 10 ml of sterile distilled water. The Petri dish was closed then held by an elastic thread and finally incubated in an oven at 30°C for 7 days [19]. The uninoculated seeds (control) were immersed in sterile distilled water.

Germination parameters evaluation

At the end of the seven (7) days of germination, the following data were collected:

- The number of seeds germinated per box was counted for the evaluation of the germination percentage [20].
- Using a graduated ruler, the length of the root and seedling of each germinated seed was measured in order to determine the vigor index, which corresponds to the sum of the average length of the roots and seedlings seed germinated from the same box multiplied by the germination percentage of the box [21].
- Using a digital scale (Highland HCB 302, Max: 300 g) of precision 0.01 g, the weights of the germinated seed (seed-seedling-roots set), seedling and roots of each germinated seed were recorded.

Data analysis

The germinal data collected were subjected to an analysis of variance with software R (3.5.3). The means were cumulated for the different treatments and compared by Student-Newman-Keuls at the 5% threshold. A principal component analysis (PCA) was performed to assess the effect of treatments on germinal parameters. The matrix used was the different measures of organs by treatment.

RESULTS AND DISCUSSION

Extraction of chitosan from exoskeletons of two species of crabs (*Callinectes amnicola* and *Cardisoma armatum*).

The Figure 1 shows the appearance of the products obtained after each step of the chitosan extraction.

[a] *Cardisoma armatum*

[b] *Callinectes amnicola*

[c] Separation of shell

[d] Drying of exoskeletons

[e] Powder obtained after grinding the exoskeletons of *C. armatum*

[f] Powder obtained after grinding the exoskeletons of *C. amnicola*

[g] Chitin obtained after demineralization and deproteination

[h] Chitin obtained after bleaching

[i] Chitosan obtained after deacetylation

Figure 1. Process of extracting chitin and chitosan from crab shells

The yields obtained vary depending on the species of crab and the stages of extraction. The results obtained from each extraction step as well as the deacetylation step are compiled in table 1. Whatever the species of crab used, the lowest yields (18 ± 0.01% and 32.11 ± 0.40%) were obtained at the demineralization stage respectively for the species *C. armatum* and *C. amnicola*. Furthermore, the ratio of the mass of bleached chitin and that of the deacetylated product shows that the chitosan yields vary from 85.73 ± 1.37% (*C. amnicola*) to 92 ± 1.50% (*C. armatum*). However, the ratio of the mass of powdered crab shells and that of the chitosan obtained showed that the chitosan

yields varied from 12.48% (*C. amnicola*) to 5.572% (*C. armatum*). These results show that it takes a good amount of powder to have a reasonable amount of chitosan. These results obtained for the *C. amnicola* species (12.48%) are close to the 10% chitosans obtained by Tolaimate et al. [22] in both red crab and marbled crab. These results are also superior to those of Demir et al. [16], who obtained a yield of 11.73% of chitin from powdered crabs of the species (*Callinectes sapidus*) and 77.78% of chitosan depending on the mass of chitin with the shells of *Callinectes sapidus*. These results are also superior to the 76% chitosan obtained from chitin with the species *Callinectes sapidus* by Kaya et al. [23] in turkey. The results obtained in this study showed the high chitosan content of *C. amnicola* and *C. armatum* crabs in the study area. As for the work of Abdou et al. [24], by extracting chitin from six different sources, these authors found 16.73% in crab shells.

Table 1: Chitin and chitosan yields at each stage of the extraction

Crab species	Parameter	Demineralization	Chitin		Chitosan	Overall yield of chitosan
			Deproteination	Bleaching	Deacetylation	
Callinectes amnicola	Powder mass (g)	500	160.55	76.03	72.82	-
	Product mass (g)	160.55±2.01	76.03±0.16	72.82±0.97	62.42±0.99	-
	Yield (%)	**32.11±0.40**	**47.36±0.10**	**95.78±1.27**	**85.73±1.37**	**12.48**
Cardisoma armatum	Powder mass (g)	500	90	32.4	27.86	-
	Product mass (g)	90±0.08	32.4±0.78	27.86±0.49	25.63±0.41	-
	Yield (%)	**18±0.01**	**36±0.87**	**86±1.51**	**92±1.50**	**5.56**

In vitro effects of chitosans on germination of maize seeds

This study allowed us to observe after seven (7) days of germination a good growth of the seedlings and roots of the seeds treated with chitosan compared to the controls (Figure 2).

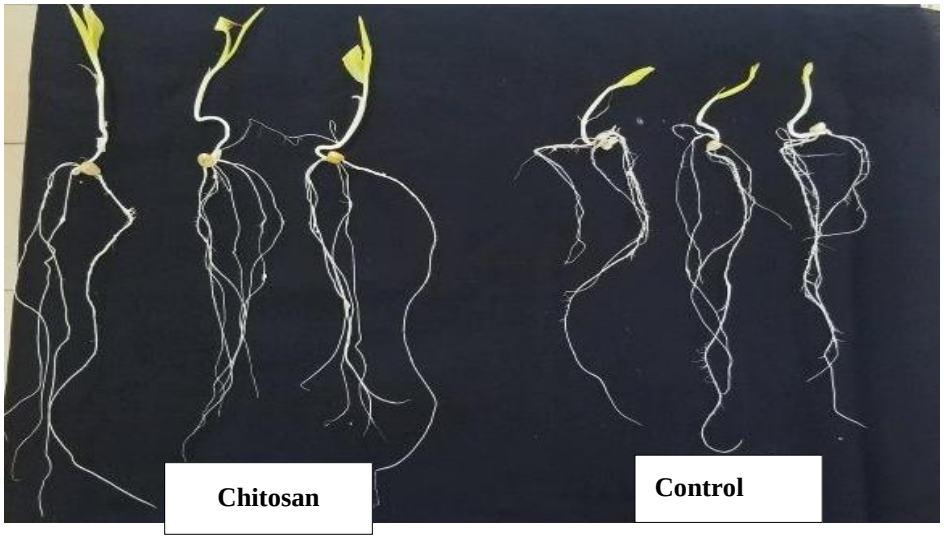

Figure 2. Appearance of the roots /seedlings of sprouted seeds

Chitosan: seeds treated with chitosan; Control: seeds without chitosan

In vitro effects of chitosans on germination of corn seeds

Table 2 shows the effects of chitosans on germination rate, root and seedling length, and vigor index. The good growth of seedlings and roots obtained is indicated by the manifestation of the biostimulant potential of the bioproducts under study.

Table 2. Effects of chitosan on germination rate, length of root, seedling and vigor index of corn seeds

Treatments	Germination rate (%)		Root Length (cm)		Seedling length (cm)		Vigor index	
	m	sd	m	sd	m	sd	m	sd
Control	97.222^{a}	4.303	22.281^{b}	0.522	12.459^{c}	0.683	3381.23^{b}	252.766
CALI 0.05	94.444^{a}	4.303	25.036^{a}	1.996	15.809^{ab}	0.773	3851.92^{a}	128.672
CALI 0.5	94.444^{a}	4.303	26.601^{a}	0.97	16.398^{a}	1.241	4057.75^{a}	121.635
CARDI 0.05	97.222^{a}	4.303	25.157^{a}	1.627	14.149^{bc}	0.265	3649.22^{ab}	203.456
CARDI 0.5	97.22^{a}	1.306	27.1380^{a}	0.663	14.755^{ab}	0.63	4076.71^{a}	289.47
Significant	ns		**		*		*	

ns = p> 0.05 (not significant); * = p <0.05 (significant); ** = p <0.01 (highly significant); In the same column, the means stamped with different letters are significantly different at the 5% level according to the Student Newman-Keuls test. m: average; sd: Standard deviation, CALI: Chitosan extracted from *Callinectes amnicola*, CARDI: Chitosan extracted from *Cardisoma armatum*.

Germination rate

There is a good improvement in the different treatments on in vitro germination of maize and the parameter values vary from one treatment to another. In fact, all the treatments including the control induced good germination of the seeds ranging from

94.44% to 97.22% (table 3). However, the difference in effect observed was not significant between the treatments. This result confirms that found by Kananont et al. [25] and Lizárraga-Paulin et al. [26]. Indeed, these authors observed that there was no difference between the germination rate of seeds of *Dendrobium bigibbum* var. Compactum and *Zea mays* l. with chitosan. However, Costales-Menéndez and Falcón-Rodríguez [27] found stimulatory effects of chitosan concentration on in vitro soybean growth. Martins et al. [28] found a significantly high germination rate in two corn hybrids when exposed to high concentrations of chitosan (600, 1200 and 2400 ppm). Guan et al. [29], they found that priming with chitosan improves the germination rate and the growth of roots and seedlings in two varieties of corn at a temperature of 15°C.

Root length

Concerning the length of the roots, all the lengths of the roots of the seeds under the influence of the various treatments are longer than those of the controls. The seeds treated with chitosan extracted from *C. armatum* at a concentration of 0.5 g / l gave the longest roots (27.13 cm) compared to the control seeds (22.28 cm) with an increase of 21.80 %. These are followed by seeds treated with chitosan extracted from *C. amnicola* at a concentration of 0.5 g / l and that extracted from *C. armatum* at a concentration of 0.05 g / l for respective increases of 19.39% and 12.91%. The observed difference in effect ($p < 0.01$) was highly significant between the treatments (table 3). These results are similar to those obtained by Costales-Menéndez and Falcón-Rodríguez[27] on soybeans with length increases ranging from 28.58 to 81.46%.

Seedling length

As for the length of the seedlings, all the seeds treated with the two types of chitosan (based on *C. amnicola* and *C. armatum*) showed better elongation of the seedlings compared to the control seeds. The highest length was obtained by seeds treated with chitosan extracted from *C. amnicola* at 0.5 g/l (16.398 cm), i.e. an increase of 31.71% followed by seeds treated with chitosan extracted from *C. amnicola* at 0.05g / l (15.81 cm), i.e. an increase of 26.98% compared to the control seeds (12.45 cm). The difference in effect observed was significant ($p < 0.05$) between the treatments. These results are comparable to those of Costales-Menéndez and Falcón-Rodríguez [27] who worked on the effect of the molecular mass of chitosan on the germination and in vitro growth of soybeans. Indeed, these authors found increases in soybeans ranging from 30.36 to 65.60%.

Vigor index

The combination of the germination rate, seedling length and root length parameters allowed us to calculate the corn seed vigor index per treatment. All treatments significantly improved the corn seed vigor index compared to controls. The seeds treated with chitosan extracted from *C. armatum* at 0.5 g / l induced good vigor (4076.71), ie an increase of 20.57% compared to the control seeds. This vigor is also

noticeable with seeds treated with chitosan extracted from *C. amnicola* at 0.5 g / l (20.01%). The observed difference in effect was significant ($p < 0.05$) between the treatments. This is because chitosan stimulates the growth of plants such as wheat [30] and *Dendrobium* [31]. Likewise, it increases photosynthesis, germination, plant vigor, promotes and improves plant growth, stimulates nutritional intake [32]. The work of Agbodjato et al. [33] has also shown the improving effect of chitosan in combination with rhyzobacteria on the seed industum. Indeed, these authors have shown that the combination of chitosan + *Azospirillium lipoferum* + *Pseudomonas fluorescens* gave the best vigor index with an improvement of 36.44% compared to the controls [33].

Effects of chitosans on the weights of roots, seedlings and germinated seed

The results of the evaluation of the effects of the chitosans produced in Benin on the weights of the roots, the seedling and the germinated seed are presented in Table 3. The analysis of this table shows that the treatments induced a good performance of the seed, weight of roots, seedlings including germinated seed.

Table 3. Effects of chitosan on in vitro germination of maize

Treatments	Root weight (g)		Seedling weight (g)		Germinated seed weight (g)	
	m	sd	m	sd	m	sd
Control	0.541	0.106	0,637	0.049	1.507	0.117
CALI 0.05	0.740	0.018	0,610	0.069	1.717	0.030
CALI 0.5	0.743	0.083	0,720	0.067	1.725	0.026
CARDI 0.05	0.679	0.061	0,726	0.021	1.651	0.053
CARDI 0.5	0.772	0.039	0,777	0.011	1.780	0.043
Significant	**		*		ns	

ns = $p > 0.05$ (not significant); * = $p < 0.05$ (significant); ** = $p < 0.01$ (highly significant); In the same column, the means stamped with different letters are significantly different at the 5% level according to the Student Newman-Keuls test. m: average; sd: Standard deviation, CALI: Chitosan extracted from *Callinectes amnicola*, CARDI: Chitosan extracted from *Cardisoma armatum*.

Therefore, the treatment, which induced the highest root weight, is that of seeds treated with *C. armatum* at 0.5 g / l, i.e. an increase of 42.70%, followed by seeds treated with chitosan extracted from *Callinectes amnicola* at 0.5, an increase of 37.34%. The difference in effect observed was significant ($p < 0.05$) between the different treatments. As for the weight of the seedling, the best result was obtained with the seeds treated with *C. armatum* at 0.5 g / l and 0.05 g / l compared to the control seeds, i.e. respective increases of 22% and 14 % compared to control seeds. The difference in effect observed was significant ($p < 0.05$) between the different treatments. Asghari et al. [34] obtained with solutions of low concentrations (5 and 15 mg / l) of chitosan, in vitro, significant

increases in fresh roots and dry matter of seedlings while high concentrations such as 500 mg / They significantly lowered the fresh weight of seedlings in vitro without affecting the dry weight of the roots.

Regarding the weight of the germinated seed, note also that the highest weight of the germinated seed is obtained by the treatment of *C. armatum* at 0.5 g / l for an increase of 18.11% compared to the controls. The difference in effect observed was significant ($p < 0.05$) between the different treatments.

It should be noted that chitosan is also recognized as a protective film for fruits and vegetables, as an antifreeze film and as a stimulator of flowering and fruiting in plants [35].

Principal component analysis of germination parameters

The Principal Component Analysis (PCA) of the germinal parameters shows that the first two axes express 96.86% of the starting information. Indeed, the projection of these parameters evaluated on the PCA axes shows that the length and weight of roots as well as the length of seedlings, the weight of germinated seeds and the vigor index are correlated with axis 1 while the germination rate and the weight of the seedlings are correlated with axis 2. Furthermore, *C. amnicola* 0.05 g / l, *C. amnicola* 0.5 and *C. armatum* 0.5 g / l are correlated with axis 1 while the *C. armatum* treatment is correlated with axis 2. Consequently, the *C. amnicola* 0.05 g / l, *C. amnicola* 0.5g / l and *C. armatum* 0.5 g / l treatments significantly improve length and weight of roots as well as length of seedlings, weight of sprouted seeds and vigor index. On the other hand, the *C. armatum* treatment 0.05 g / l significantly improves the germination rate and the weight of the seedlings (Figure 3).

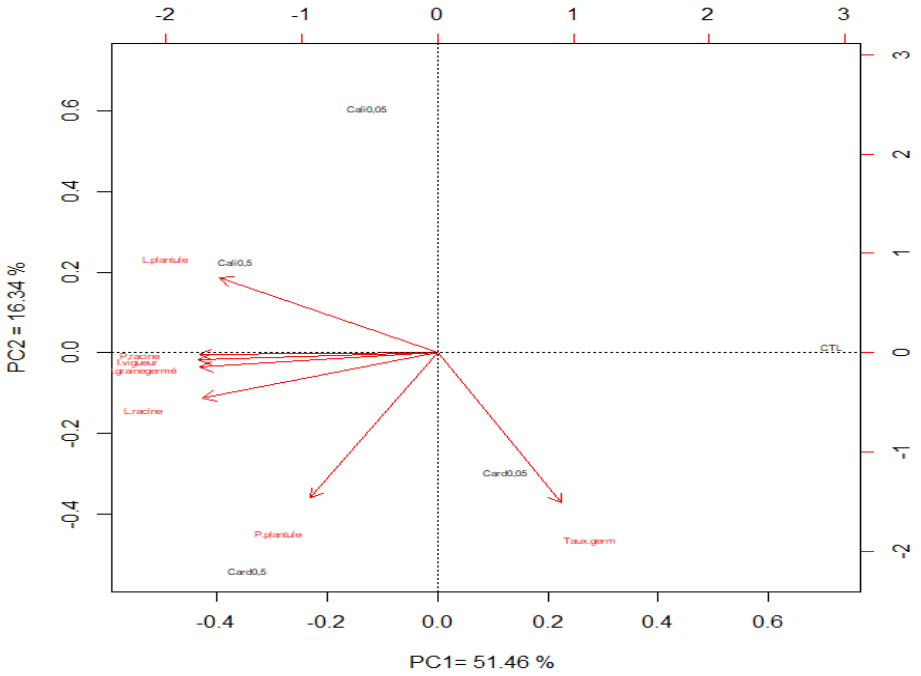

Figure 3. Principal component analysis on germinal parameters

In general, Morin-Crini et al. [36] reported that in the coated form, chitosan protects plants such as cotton, tomato, and wheat and has positive effects on the germination rate, growth parameters and yield of different crops such as soybeans, ornamentals, corn, wheat, lentil, rice and peanuts. The same is true for Cheba [37] who reported, by several authors, the effects of chitosan in coated form as a fertilizer, biopesticide and fungicide, growth enhancer, stimulator of the hormones responsible for the formation of roots, the growth of stems and fruit development. Malerba and Cerana [38] also reported the growth promoting effects of chitosan in corn after seed coating or plant spraying. In foliar application, Farouk and Amany [39] found that chitosan at 250 mg / l significantly improved all the growth parameters in the pea *Vigna unguiculata* (L) Walp.

CONCLUSSION

This study shows the availability of chitin and chitosan in the shells of *C. amnicola* and *C. armatum* crabs in Benin. Thus, the application of chitosan revealed an improvement in the germination parameters of corn seeds. The best vigor index was obtained with chitosan extracted from *C. armatum* at 0.5g / l, as well as the longest root length. However, the highest seedling length was obtained with chitosan extracted from *C. amnicola* at 0.5 g / l. The study proves the possibility of the use of chitosan as a biostimulant in agricultural production in Benin. However, experiments in a controlled environment and in a real environment are necessary to confirm the expression of the best chitosans on the recorded grain yields.

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REFERENCES

[1]. Faessel, L., and Tostivin, C., 2016, Les produits de stimulation en agriculture : un état des connaissances. Notes et Etudes Socio-Economiques, 12(40), 7-39.
[2]. Boonlertnirum, S., Meechoul, S., and Sarobol, E., 2010, Physiological and morphological responses of field corn seedlings to chitosan under hypoxic conditions. Science Asia 36 (2010), 89-93.
[3]. Bornet, A., and Teissedre, P.L., 2005, Intérêt de l'utilisation de chitine, chitosane et de leurs dérivés en œnologie, Journal International des Sciences de la Vigne et du vin, 39 (4), 199-207.
[4]. Katiyar, D., Hemantaranjan, A., Singh, B., and Bhanu, A.N., 2014, A future perspective in crop protection: chitosan and its oligosaccharides. Advances in Plants and Agriculture Research, 1(1), 1-8.

[5]. Sharif, R., Mujtaba, M., Ur Rahman, M., Shalmani, A., Ahmad, H., and Anwar, T., 2018, The multifunctional role of chitosan in horticultural crops; a review. Molecules. 23(4), 872.

[6]. Mondal, M., Puteh, A.B., and Dafader, N.C., 2016, Foliar application of chitosan improved morphophysiological attributes and yield in summer tomato *Solanum lycopersicum*. Pakistan Journal of Agricultural Sciences. 53(2), 339-344.

[7]. Zhang, X., Li, K., Xing, R., Liu, S., and Li, P., 2017, Metabolite profiling of wheat seedlings induced by chitosan: Revelation of the enhanced carbon and nitrogen metabolism. Frontiers in plant science, 8(2017), 1-13.

[8]. Rurangwa, E., van den Berg, J., Laleye, P., van Duijn, A.P., and Rothuis A.J., 2014, *Mission exploratoire Peche, Pisciculture et Aquaculture au Benin: un quick scan du secteur pour des possibilities dínterventions* (No. C072/14). IMARES. https://library.wur.nl/WebQuery/wurpubs/fulltext/308953

[9]. Tohozin, Y.A., 2012, Pêcheries sédentaires, production et commercialisation des tourlourous des lagunes (*Cardisoma armatum*), des crabes bicornes (*Callinectes amnicola*) et des étrilles lisses (*Portunus validus*) dans l'ouest du lac Nokoué en République du Bénin. Journal de la Recherche Scientifique de l'Université de Lomé (Togo), 14(1), 12 -21.

[10]. Hinvi, L.C., Sohou, Z., Agadjihouedé, H., Laleye, P., and Sinsin, B., 2013, Domestication de *Portunus validus* et *Callinectes amnicola* au Bénin, Journal de la Recherche Scientifique de l'Université de Lomé (Togo), série (A), 15(2), 13-22.

[11]. Dessouassi, C.E, Chikou, A., Lederoun, D., Adandedjan, D., Gangbe, L., and Laleye, P., 2018, Diversity, biology and exploitation of brackish water crabs in West Africa: A review, International Journal of Biological and Chemical Sciences, 12(5), 2355-2370.

[12]. Goussanou, A., Chikou, A., Ogni, A.C., Kassa, S.K., Houessionon, B., Mensah, G.A., and Youssao, A.K.I., 2017, Synthèse des connaissances sur l'écologie et la biologie des crabes (Crustacés, Décapodes, Brachyoures), International Journal of Biological and Chemical Sciences, 11(6), 2990-3004,

[13]. Jacob S. (2017). Valorisation des déchets: La science se penche sur le duo crabe-crevette. Edition N°:5054, https://www.leconomiste.com , consulté le 05/07/2018.

[14]. Kandra, P., Challa, M.M., and Jyothi, H.K., 2012, Efficient use of shrimp waste: present and future trends, Apply in Microbiology and Biotechnology, 93(1), 17-29.

[15]. Oanh, T., Robert, H., Frederic, M., and Patrick, N., 2007, Valorisation des résidus industriels de pêches pour la transformation de chitosane par technique hydrothermo-chimique. Journal of Water Science, 20(3), 253-262.

[16]. Bölgen, N., Demir, D., Öfkeli F, and Ceylan S., 2016, Extraction and Characterization of Chitin and Chitosan from Blue Crab and Synthesis of Chitosan Cryogel Scaffolds. Journal of Turkish Chemical Society Section A: Chemistry, 3(3), 131-144.

[17]. Yallou, C.G., Aïhou, K., Adjanohoun, A., Toukourou, M., Sanni, O.A., and Ali, D., 2010, Itinéraires techniques de production de maïs au Bénin. Fiche technique. Dépôt légal N° 4922 du 3 Décembre, Bibliothèque Nationale du Bénin, 18 p.

[18]. Gholami, A., Shahsavani, S., and Nezarat, S., 2009, The effect of Plant Growth Promoting Rhizobacteria (PGPR) on germination, seedling growth and yield of maize. International Journal of Biology and Life Sciences, 1, 35-40.

[19]. Neelesh K, Arvind A, Hirdesh K, and Asad A. (2011). Physiological and biochemical changes during seed deterioration in aged seeds of (*Zea mays L.*), American Journal of Plant Physiology, 6(1), 28-35.

[20]. Khayatnezhad, M., and Gholamin, R., 2011, Effects of salt stress levels on five maize (*Zea mays* L.) cultivars at germination stage. African Journal of Biotechnology, 10(60), 12909-12915.

[21]. Abdul-Baki A. and Anderson J. D., 1973, Vigor determination in soybean seed by multiple criteria, Crop Science, 13(6), 630–633.

[22]. Tolaimate, A., Rhazi, M., Alagui, A., Desbrieres, J., and Rinaudo, M., 2008, Valorisation des déchets des industries de pêche par production de la chitine et du chitosane. Physical and Chemical News 42 (2008), 120-127.

[23]. Kaya, M., Dudakli, F., Asan-Ozusaglam, M., Cakmak, Y.S., Baran, T., Mentes, A., and Erdogan, S., 2016, Porous and nanofiber a-chitosan obtained from blue crab (*Callinectes sapidus*) tested for antimicrobial and antioxidant activities. LWT - Food Science and Technology, 65, 1109-1117.

[24]. Abdou S. E., Nagy S.A.K., and Elsabee Z. M., 2008, Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology 99(5), 1359–1367

[25]. Kananont, N., Pichyangkura, R., Chanprame, S., Chadchawan, S., and Limpanavech, P., 2010, Chitosan specificity for the *in vitro* seed germination of two *Dendrobium orchids* (Asparagales: Orchidaceae). Scientia Horticulturae, 124 (2), 239–247.

[26]. Lizárraga-Paulín, E.G., Torres-Pacheco, I., Moreno-Martínez, E., and Miranda-Castro, S.P., 2011, Chitosan application in maize (*Zea mays*) to counteract the effects of abiotic stress at seedling level, African Journal of Biotechnology, 10(34), 6439-6446.

[27]. Costales-Menéndez, D., and Falcón-Rodríguez, A.B., 2020, Effect of chitosan molecular mass on germination and *in vitro* growth of soy. Cultivos Tropicales, 41(1), e05 http://ediciones.inca.edu.cu

[28]. Martins, M., Veroneze-Júnior, V., Carvalho M., Carvalho, D.T., Barbosa, S., Doriguetto, A.C., Magalhães, P.C., Ribeiro, C., dos Santos, M.H., and de Souza, T.C., 2018, Physicochemical characterization of chitosan and its effects on early growth, cell cycle and root anatomy of transgenic and non-transgenic maize hybrids. Australian Journal of Crops Sciences, 12(01), 56-66

[29]. Guan, Y.J., Hu, J., Wang, X.J., and Shao, C.X., 2009, Seed priming with chitosan improves maize germination and seedling growth in relation to physiological changes under low temperature stress. Journal of Zhejiang University, Science B, 10(6), 427–433.

[30]. Tham, L. X., Nagasawa, N., Matsuhashi, S., Ishioka, N.S., Ito, T., and Kume T., 2001, Effect of radiation-degraded chitosan on plants stressed with vanadium, Radiation Physics and Chemistry 61(2), 171–175.

[31]. Nge, K.L., New, N., and Chandrkrachang, W., 2006, Chitosan as a growth stimulator in orchid tissue culture, Plant Sciences, 170(6), 1185-1190.

[32]. Hernández, Y. B., 2004, La quitina y la quitosana, polisacáridos animales de gran importancia Ciudad Habana Cuba, 7p.

[33]. Agbodjato, A.N., Noumavo, P.A., Adjanohoun, A., Agbessi, L., and Baba-Moussa L., 2016, Synergistic Effects of plant Growth Promoting Rhizobacteria and Chitosan on *in vitro* seeds Germination, Greenhouse growth, and nutrient uptake of Maize (*Zea mays* L.), Biotechnology Research International, 2016, 1-11.

[34]. Asghari-Zakaria, R., Maleki-Zanjani, B., and Sedghi E., 2009, Effect of *in vitro* chitosan application on growth and mini tuber yield of *Solanum tuberosum* L, Plant Soil Environment, 55(6), 252–256.

[35]. Krajewska, B., 2005, Application of chitin and chitosan-based materials for enzyme immobilizations: a review, Enzyme and Microbial Technology, 35(2-3), 126-139.

[36]. Morin-Crini, N., Lichtfouse, E., Torri, G., and Crini, G., 2019, Applications of chitosan in food, pharmaceuticals, medicine, cosmetics, agriculture, textiles, pulp and paper, biotechnology, and environmental chemistry, Environmental Chemistry Letters, 17 (4), 1667-1692.

[37]. Cheba, B. A., 2011, Chitin and Chitosan: Marine Biopolymers with Unique Properties and Versatile Applications, Global Journal of Biotechnology and Biochemistry, 6 (3), 149-153.

[38]. Malerba, M., and Cerana, R., 2018, Recent Advances of Chitosan Applications in Plants, Polymers 10(2), 118.

[39]. Farouk, S., and Amany, A. R., 2012, Improving growth and yield of cowpea by foliar application of chitosan under water stress, Egyptian Journal of Biology, 14, 14-26