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EFFICACY OF CHITOSAN EXTRACTED FROM CRAB EXOSKELETONS (CALLINECTES AMNICOLA AND CARDISOMA ARMATUM) IN COMBINATION WITH PSEUDOMONAS PUTIDA ON THE GROWTH AND GRAIN YIELD OF MAIZE ON FERRALLITIC SOIL IN SOUTH BENIN

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ABSTRACT

The objective of the study was to evaluate chitosans produced from the exoskeletons of water (*Callinectes amnicola*) and land (*Cardisoma armatum*) crabs for use in agriculture in Benin. Then, the effects of these chitosans were combined with Pseudomonas putida in order to see their synergistic effects on the growth and yield of maize seeds of the variety EVDT 97 STR C1 for 80 days. The experimental design was a block of 13 treatments with three (03) replicates. After 60 DAS in the field, application of the combination *C. amicola* + *P. putida* + 50% NPK and *C. armatum* + *P. putida* + 50% NPK showed the highest average heights. Plants treated with the combination of *C. armatum* + *P. putida* + 50% NPK and *C. amnicola* + 50% NPK gave the best corn grain yields with increases of 51.68% and 45.57% respectively. This study confirms that sources of chitosan from shellfish exoskeletons are available in Benin and shows the potential to use

chitosan alone or in combination with Rhizobacteria as bio fertilizers to improve productivity and increase maize yield in Benin while reducing the use of chemical fertilizers.

1. INTRODUCTION

Faced with the ever-increasing population of Africa and West Africa and the resulting growing food needs, increasing yields in agriculture has become a major challenge. However, the agricultural sector is facing problems, one of the most important of which is the degradation of soil fertility. The overuse of external inputs such as mineral fertilizers and pesticides can reduce considerable increases in food production but also reduce soil fertility and its biological components [1]. This also has adverse consequences for human health. In response to this problem, improving soil fertility is one of the common strategies for increasing agricultural production. Thus, it becomes important to develop different biological control methods using natural organisms to reduce the effects of a harmful organism.

The appearance on the market of agricultural inputs 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 is arousing the interest of actors in the agricultural world [2]. These substances are called bio fertilizers and among them are chitin and chitosan. Chitosan is a biodegradable substance of natural origin obtained by the deacetylation of chitin, which is found, among others, in the exoskeletons of crustaceans such as lobsters, shrimps and crabs [3]. In Benin, work on chitosan applications is very recent and promising [4],[5].

The use of chitosan, in spite of the very interesting results obtained worldwide in the fields of agricultural yield improvement [6],[7],[8],[9] is not accessible to the majority of producers in poor countries because of its price, which ranges from 23 to 9160 euros/kg depending on the quality[10]. Chitosan is a high value-added product obtained from a cheap raw material [10]. According to 2003 data, the world catches of crabs are estimated at 1.2 million t/year [11]. However, it should be pointed out that since crab is a coastal animal that is very easy to catch, this figure does not take into account the individual and artisanal fisheries, especially in poor countries where crab represents an important source of animal protein [12]. Benin produces a significant quantity of crabs annually. Indeed, according to Benin Agricultural Ministry data, in its 2016-2018 Management Program Budget, crab production should increase from 3637.19 tons in 2013 to 6639.19 tons in 2018. According to Rurangwa et al. [12], 70% of Benin's total crab production is exported to Togo and Ghana. Despite the growing human consumption of these products, the huge quantities of waste generated are simply dumped into the marine environment or into public dumpsites, creating serious pollution problems in the process. The biodegradation of shells of crustaceans is very slow [13]. In the need to process and use, crab waste that contains several bioactive compounds such as chitin [3],[14],[15] showed the availability of crabs and based on different production procedures of chitosan found in the literature [3]. Mikpon et al. [16] have extracted chitosan from exoskeletons of water crabs (*Callinectes amnicola*) and land crabs (*Cardisoma armatum*) collected locally in Southern Benin.

2. MATERIAL AND METHODS

2.1. STUDY AREA

The study was carried out on the experimental station of the Centre de Recherches Agricoles Sud (CRA-Sud) of INRAB, located in Niaouli in the commune of Allada (Figure 1). The experimental station is located at an altitude of 105°, longitude 2° 19' East and latitude 6° 12' North. The climate is of maritime sub-equatorial type with two (02) rainy seasons and two (02) dry seasons. The soil is ferrallitic, deep and without concretion [17]. The choice of site for the implementation of the trial was made taking into account the fact that the decline in soil fertility is a priority constraint. The site is flat with a maximum slope of 2% and not flooded.

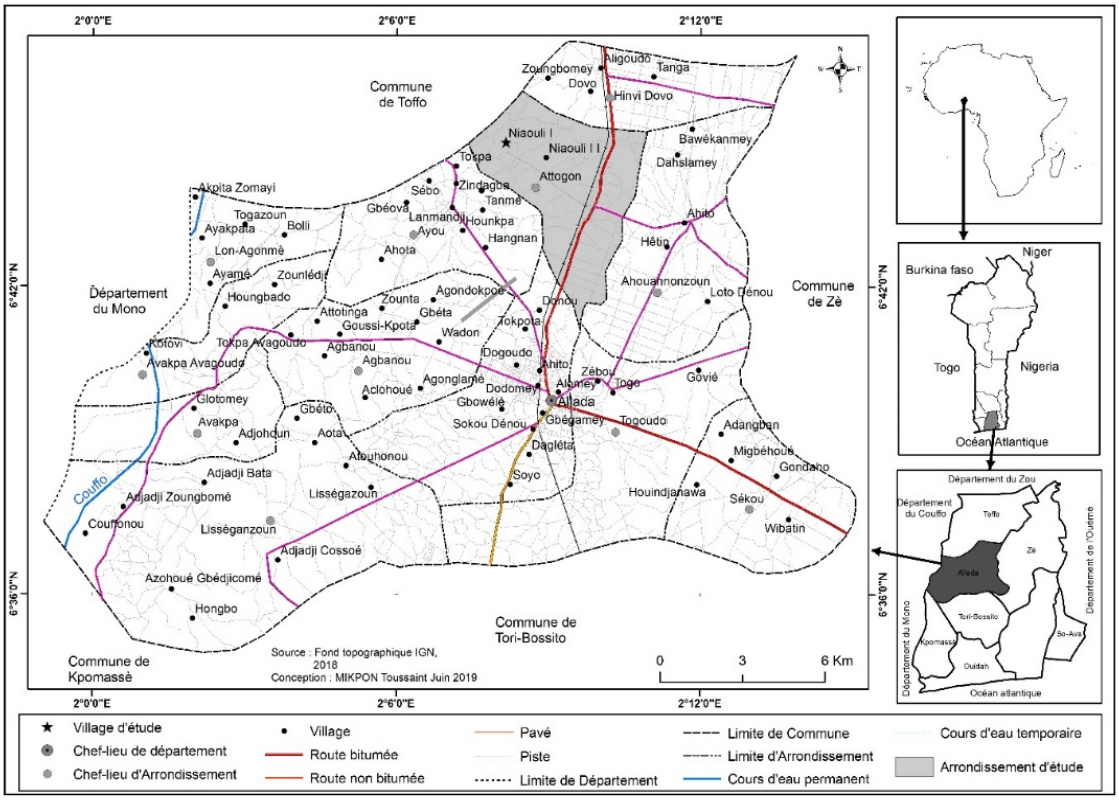


Figure 1: Geographic Location of Study Setting

2.2. BIOLOGICAL MATERIALS

The different chitosans extract respectively from exoskeletons of water crabs (*Callinectes amnicola*) and land crabs (*Cardisoma armatum*). The Rhizobacteria PGPR named *Pseudomonas putida* was used in this study to see the synergistic effect of the combination of PGPR and extracted Chitosan. This strain was isolated and identified by Adjanohoun et al.[18]and then concerted in MH broth at - 80°C at the Laboratory of Biology and Molecular Typing in Microbiology (LBTMM).

2.3. SOIL PREPARATION OF THE EXPERIMENTAL SITE

Soil preparation consisted of clearing the plot with machetes, ploughing the soil to a depth of 15 cm using a tractor attached to a disc plough and levelling the soil with a tractor attached to a harrow. The elementary plots were delimited using the "3 - 4 - 5" method.

2.4. EXPERIMENTAL DEVICE

The large plot factor was the fertilizer (NPK) dose and the modalities (Table 1) of the other two factors were combined and randomized on the small plots.

Table 1: Factors and modalities of experimental device in the field

Factors	Modalities
Types of Chitosan	Chitosan Free (C0) *Callinectes amnicola* (C1) *Cardisoma armatum* (C2)
Fertilizer dose - 0% (D0)	0 % (D0)

	50 % (D1) 100 % (D2)
Presence of bacteria *Pseudomonas putida*	Bacteria free (P0) - With bacteria (P1)

The experimental design (Figure 2) set up was a divided plot design with three replicates. The plots were divided into three large blocks representing the replicates. Each block was divided into three large plots (two had 6 small plots and one had one small plot).

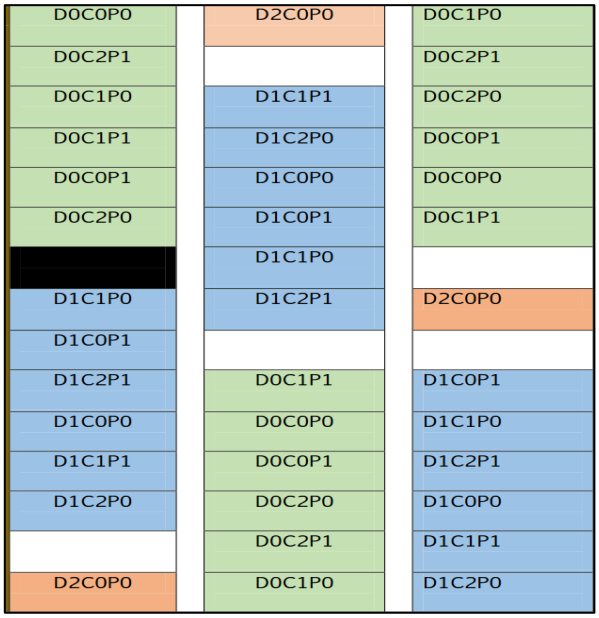


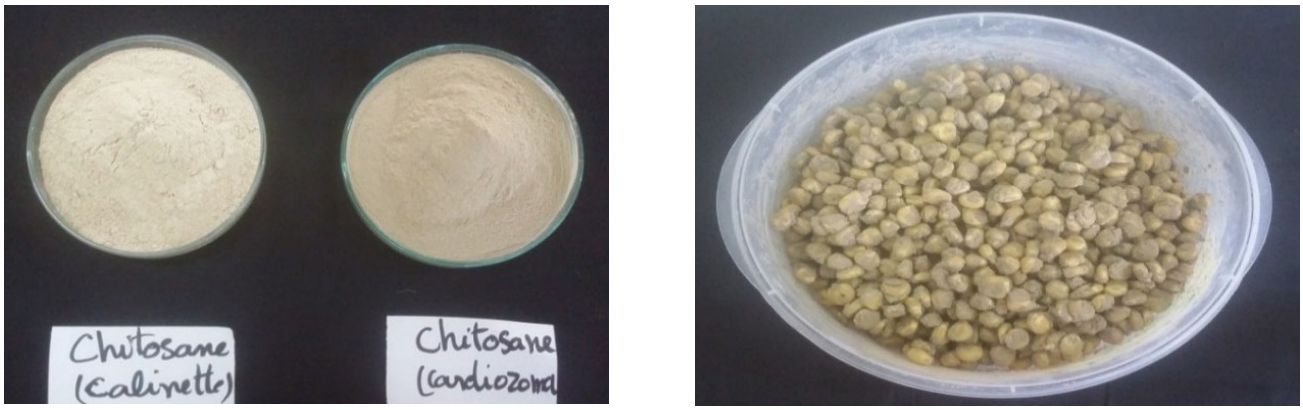
Figure 2: Diagram of experimental device

Each elementary plot has a surface area of 12.8 m^2 and is made up of 4 lines of 4 m in length with 0.80 m spacing. The distance separating the plots from each other and the repetitions separating them from each other was 1.5 m and 2 m respectively. The useful plot has an area of 6.4 m^2, on which data were collected at the two (02) central lines. The treatments evaluated are defined as follows:

T1: without chitosan Without PGPR (absolute control)
T2: 100% NPK
T3: Chitosan extracted from *Callinectes amnicola*
T4: Chitosan extracted from *Cardisoma armatum*
T5: 50% NPK
T6: Chitosan extracted from *Callinectes amnicola* +50% NPK
T7: Chitosan extracted from *Cardisoma armatum* +50% NPK
T8: *P. putida*
T9: Chitosan extracted from *Callinectes+ P. putida*
T10: Chitosan extracted from *Cardisoma armatum* + P. putida
T11 Chitosan extracted from *Callinectes + P. putida*+50% NPK
T12: Chitosan extracted from *C. armatum + P. putida*+50% NPK
T13: *P. putida*+50% NPK

2.5. CHITOSAN PREPARATION AND SEED COATING

The powders of the two crab species were used for the extraction of chitosan according to the extraction methodology. The seeds were coated in the resulting mixture. They were then dried in ambient air (Figure 3).



a **b**

Figure 3: Chitosan powder extracted from *C. amnicola and C. armatum* (a), Corn seeds coated with chitosan (b)

2.6. PREPARATION OF PGPR SUSPENSIONS

The *Pseudomonas* putida strains were revived by transplanting on agar media for 24 hours. Bacterial suspensions of PGPR were obtained by culture in nutrient medium (MH broth) for 24 hours at 30°C. Then, another culture was carried out from the previous 24 hours at a rate of 10 ml of each culture in 1500 ml of nutrient medium (MH broth). After the 24 h incubation, the culture was then adjusted to a microbial concentration of about 1 x 10^8 CFU/ml (OD 0.45 at 610 nm) with a spectrophotometer according to the method described by Govindappa et al. [19].

2.7. COATING, SOWING AND SEED INOCULATION

The sowings were made with a spacing of 0.40 m × 0.80 m at a rate of 02 seeds previously coated with chitosan per pots on June 07, 2019 with application of NPK. Two (2) coated (Figure 4 a) or uncoated corn seeds were placed in a 5 cm pot. Then according to each treatment, the corn seeds were inoculated with 10 ml of bacterial suspension of about 10^8 CFU/ml (Figure 4 b). In addition, the trial was protected by nets to prevent the destruction of the plants by animals.

a **b**

Figure 4: (a): coated seeds, (b): sowing and inoculation

2.8. EVALUATION OF GROWTH AND YIELD PARAMETERS IN THE FIELD

Parameters related to the growth of corn plants

Height and diameter at the collar of the plants were measured every fifteen (15) days using tape measures and calipers respectively from the time of plant emergence until 60 days after sowing (DAS). Data for the calculation of plant leaf area will be measured only at 60 DAS [20].

Plant biomass and maize grain yield parameters

The maize yield data collected were plant biomass and maize kernel weight. The biomass produced was determined at harvest. Thus, for each elementary plot, 20 maize plants were uprooted, cut into small pieces and mixed in a bucket. The mixture was placed in a labelled husk, which was placed in an oven at 100°C for 72 hours, during which time the husk was regularly removed from the oven and weighed, using a precision scale, until the weight was completely stabilized.

Corn grain yield was determined as follows: the cobs of six (06) corn plants were harvested from two (02) center rows of each elementary plot, despatched and shelled. The obtained maize grains were dried in an oven at 65°C for 72 hours until constant weight was obtained, then the grains were weighed using a scale (Highland HCB 302, Max: 3001g) with an accuracy of 0.1 g. Grain maize yield values were obtained according to the following formula, used by Adjanohoun *et al.* [18] (2012):

$$R = \frac{P \times 10.000}{S \times 1.000}$$

Where:

- R is the maize yield, expressed in t/ha;
- P is the mass of maize per elementary area of calculation, expressed in kg;
- S is the area of the useful plot in m^2;
- 10000 is the conversion from m^2 to ha; and
- 1000 is conversion from kilogram (kg) to ton (t).

Statistical analysis of the data

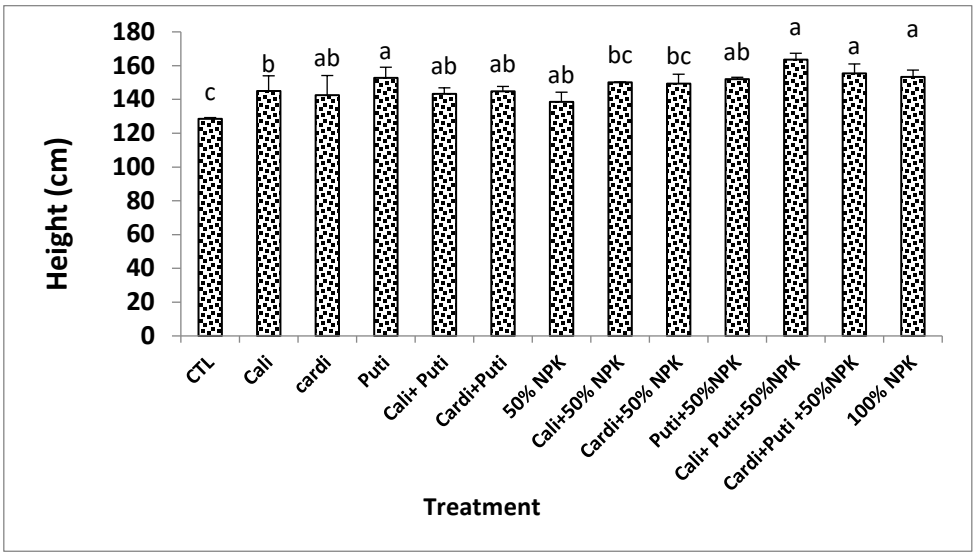
An ANOVA analysis of variance followed by mustache boxes was performed to evaluate the effect of the treatments on growth parameters. Then a PCR principal component analysis was performed to evaluate the effect of treatments on growth parameters. The matrix used was different organ measurements per treatment.

3. RESULTS AND DISCUSSION

3.1. EFFECTS OF EXTRACTED CHITOSANS ON GROWTH PARAMETERS (HEIGHT, DIAMETER, LEAF AREA)

3.1.1. HEIGHT OF THE PLANTS

The results show that after 60 DAS, a good elevation of the maize plants was observed at all treatments compared to the control. After 60 DAS, there was good elevation of maize plants in all treatments (Figure 5). This elevation is increasingly remarkable with the combination of plants treated with chitosan extracted from *C. amnicola* (163.53 cm) *or C. armatum* (155.51 cm) plus *P. putida* with 50% NPK. On the other hand, the plants treated with the combination of *C. amnicola* + *P. putida*+50% NPK *and C. armatum + P. putida*+50% NPK showed the highest average heights with increases of 27.33% and 28.09% respectively. These even surpass the plants treated with 100% NPK. On the other hand, only the untreated plants (control) gave the lowest heights (128.43 cm). The difference in effect observed was highly significant between treatments ($p < 0.01$) Figure 5. These results are similar to those obtained by Jelin et al.[21]in India on height and Agbodjato et al. [4] in Benin who also observed increases in height of 12.85% and 17% respectively following the combination of *P. putida* + chitosan + 50% NPK compared to the control.



CTL: absolute control Cali: chitosan extracted from *C. amnicola, Cardi: chitosan extracted from C. armatum; puti: P. putida*; Cali+puti: combination of chitosan extracted from *C. amnicola and P. putida*, Card+puti: combination of chitosan extracted from *C. armatum + P. putida*

Figure 5: Plant height at 60th DAS

3.1.2. DIAMETER AT THE COLLAR OF THE PLANTS

Results obtained from this study, clearly show a good development of the collar of all treated plants compared to untreated plants (absolute control). The average diameter at the highest collar was obtained with the plants having received the contribution of the combination of: chitosan extracted from *C. armatum* + *P. putida* + 50% NPK (1.71 cm) with an increase of 18% followed by the combination of: chitosan extracted from *C. armatum* + *P. putida* for an increase of 17.20%. These values show that the treatment of chitosan extracted from *C. armatum* + *P. putida* with 50% NPK boosted the development of the collar of the plants (Figure 6). The difference in effect observed was highly significant between treatments ($p < 0.01$).

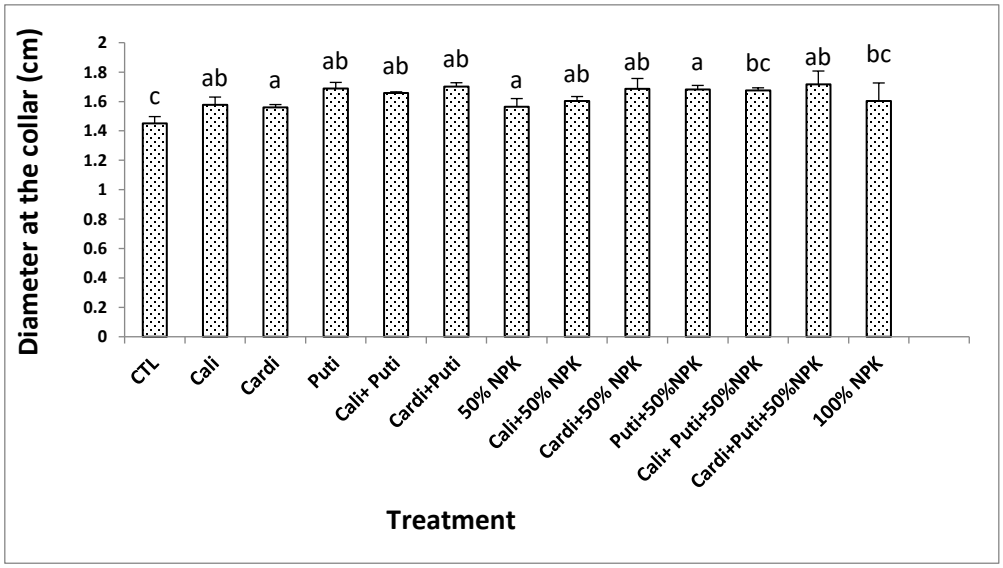


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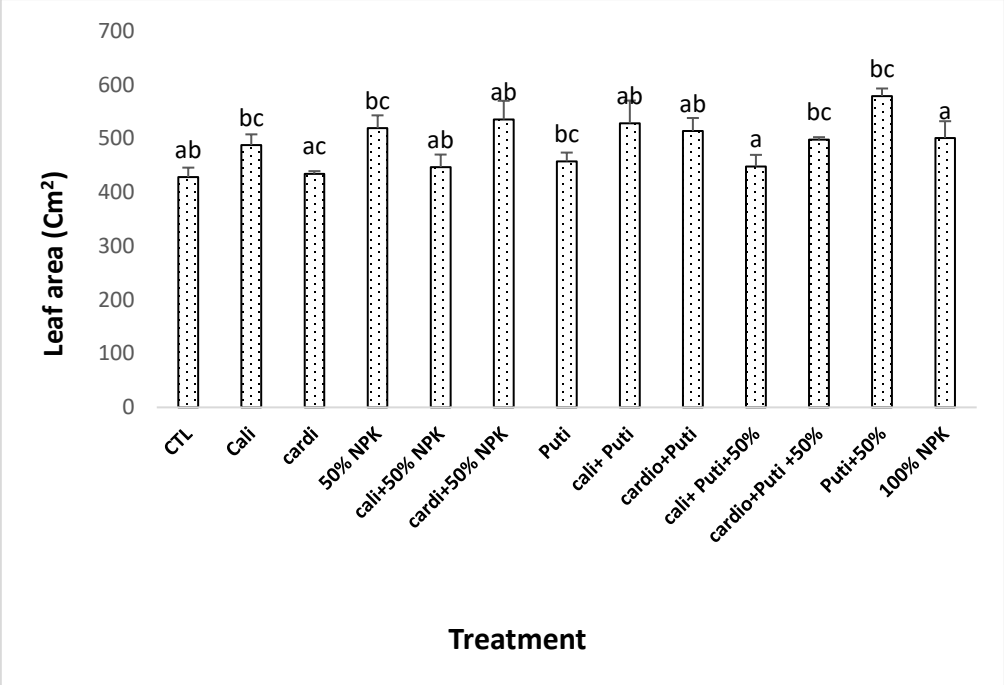
Figure 6: Plant crown diameter at 60 DAS.

3.1.3. LEAF AREA

Regarding the foliar surface, the plants treated with the combination of *P. putida* + 50% NPK (579.37 cm^2) and *C. armatum* + 50% NPK resulted in a better development of the foliar surface of the plants with increases of 35.19% and 24.97%, respectively, compared to the control. Moreover (Figure 7), the use of 50% NPK in combination with PGPR or chitosan extracted from *C. armatum* allowed to further boost the result. The difference in effect observed was significant between treatments ($p < 0.05$).

These results also confirm those of Shaharoona et al. [22] who mentioned the efficacy of Pseudomonas on increasing maize plant growth. This also explains the positive effect observed on maize plant growth and development by Walker et al.[23]. Indeed, PGPRs have direct positive effects on plant growth and yield increase of crops such as vegetables, apple, lemon, blueberry, blackberry, apricot, raspberry, sugar beet etc. [24],[25]. As for chitosan, it stimulates the plant for the synthesis of protective agents, and behaves as a fertilizer that accelerates germination and plant growth [26].



CTL: absolute control; Cali: chitosan extracted from *C. amnicola*; Cardi: chitosan extracted from *C. armatum; puti: P. putida*; Cali+puti: combination of chitosan extracted from *C. amnicola* and *P. putida, Card+puti*: combination of chitosan extracted from *C. amnicola + P. putida.*

Figure 7: Leaf area of plants at 60th DAS

Increases in plant growth parameters by PGPR and chitosan may be due to the increase in local nutrient availability, the ease of nutrient uptake by plants and the decrease in toxicity produced by heavy metals Burd et al. [27]. Several authors argue that PGPRs can promote host plant growth through various mechanisms such as nitrogen (N2) fixation and solubilization of trace elements such as phosphate (P) [25],[28],[29]. In the same vein, work carried out by Wanichpongpan et al. [30] showed the stimulating effect of chitosan on *Gerbera jamesonii* and *Gladiolus spp*. plants respectively. Hasegawa et al. [31] reported that the increase in height and diameter on onions was obtained following the cultivation of *Arisaema ternatipartitum* in a substrate with chitosan added.

3.2. EFFECTS OF EXTRACTED CHITOSANS ON YIELD PARAMETERS (PLANT BIOMASS AND GRAIN YIELD)

3.2.1. BIOMASS YIELD OF PLANTS

Table 2 presents the results of the biomass yield of the different treatments. The table shows a remarkable difference in the yield of fresh biomass above and below ground between the different treatments compared to the control. The best fresh biomass yield was obtained with the combination of *C. armatum* + *P. putida* + 50% NPK, followed by the combination of *C. armatum* + *P. putida* with increases of 18.42% and 17.20%. On the other hand, the fresh underground biomass was obtained with the combination of *C. armatum* + *P. putida* followed by the combination of *C. amnicola* + *P. putida* with increases of 93.52% and 90.74%.

With regard to the dry biomass yield, both above and below ground, it can be noted that the yield of all treated plants exceeded control. The highest yield was obtained with the combination of *C. armatum* + *P. putida* + 50% NPK for the above-ground dry biomass and the combination of *C. armatum* + *P. putida* for the below-ground dry biomass for increases of 12.12% and 67.28%, respectively.

Dobbelaere et al. [32],[33] showed that inoculation of wheat plants with *Azospirillum brasilense* induced an increase in the dry weight of the root system and the upper part of the root. In addition, Lemanceau et al. [34] claimed that bacteria of the genus Pseudomonas are able to synthesize siderophors called pyoverdines or pseudobactins. These molecules are involved in improving plant growth and health.

Table 2: Biomass yield of plants

Treatments	FAB (g)		FSB (g)		DAB (g)		DSB (g)	
	M	E	M	E	M	E	M	E
CTL	996,11	42,44	403,11	23,95	857,33	41,82	300,55	44,72
Cali	1108,01	23,72	470,33	22,84	906,11	37,65	389,44	40,27
cardi	1087,55	46,31	446,55	34,21	927,44	22,04	376,77	31,28
50% NPK	1121,88	42,40	579,26	35,71	914,7	34,10	436,01	29,22
Cali+50% NPK	1022,77	27,29	623,33	23,85	911,33	34,23	483,55	20,17
Cardi+50%	1155,33	27,33	678,11	29,68	946,00	34,93	487,55	25,50
NPK Puti	1143,77	34,96	696,22	28,51	953,44	32,75	497,77	28,33
cali+ Puti	1154,22	12,00	768,88	16,42	959,77	40,35	399,88	13,09
cardi+Puti	1167,44	29,83	780,11	18,03	955,55	31,59	502,77	38,44
cali+ Puti+50%	1114,22	20,45	602,66	29,13	955,44	39,67	485,66	38,75
cardi+Puti +50%	1179,66	41,74	612,55	41,41	961,22	35,85	435,33	31,81
Puti+50%	1118,88	26,26	595,11	35,04	960,11	46,74	444,55	31,73
100% NPK	1128,55	65,41	626,98	22,96	960,744	34,81	451,66	32,42
Signification	**		***		**		**	

*** = p < 0.001 (highly significant) ; ** = p < 0.01 (highly significant) ; In the same column, the means struck by the different letters are significantly different at the 5% threshold according to the Student Newman-Keuls test. M : mean; e: Standard deviation, Cali: Chitosan extracted from *C. amnicola*, Card: Chitosan extracted from *C. armatum*; CTL: control; puti: *P. putida*; Cali+puti: combination of Chitosan extracted from *C. amnicola* and P. putida, Card+puti: combination of Chitosan extracted from *C. amnicola* + *P. putida*. FAB : Fresh Aerial Biomass, FSB : Fresh subterranean Biomass, DAB : Dry Aerial Biomass; FSB: Fresh Subterranean Biomass.

3.2.2. CORN GRAIN YIELD

Figure 8 shows the corn grain yield results. There was good yield performance in all treatments compared to the control plants. The best yield was induced by the combination of chitosan extracted from *C. armatum + P. putida* +50% NPK (2.71 T/ha) followed by that of chitosan extracted from *C. amnicola* +50% NPK (2.63 T/ha) with respective increases of 51.68% and 45.57% compared to the absolute control. It should be noted that the 50% NPK in combination with the chitosan extracted from *C. armatum + P. putida* allowed to increase this yield more and more. The difference in effect observed was highly significant between treatments (p < 0.01).

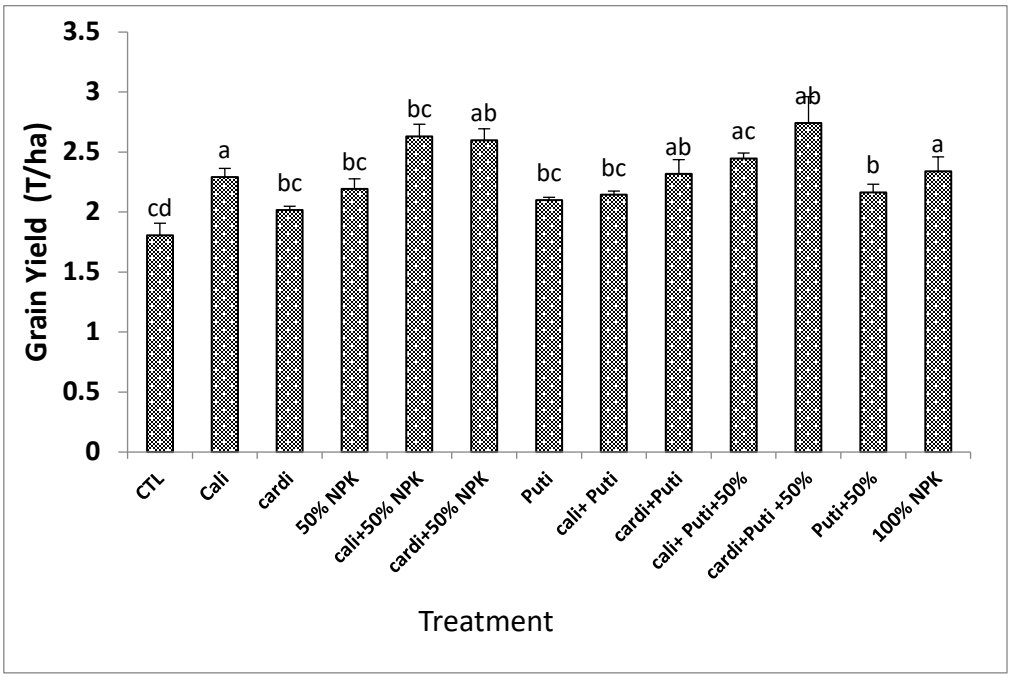


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Figure 8: Variation in Maize Grain Yield

4. CONCLUSION

The combined effects of chitosan obtained from local crab exoskeletons (*C. amnicola* and *C. armatum*) with *Pseudomonas putida* had a positive effect on maize plant growth parameters such as height, diameter at the collar and leaf area on ferrallitic soil in southern Benin. Good yield performance (plant biomass and grain yield) was obtained in all treatments compared to the control plants. The combination of chitosan extracted from *C. armatum* + *P. putida* + 50% NPK (2.7 T/ha of maize) and chitosan extracted from *C. amnicola* + 50% NPK (2.6 T/ha of maize) resulted in respective increases of 51.7% and 45.6% compared to the absolute control. These results augur well for the possibility of using extracted chitosan in combination with *P. putida* to improve maize productivity in South Benin while reducing the recommended fertilizer dose.

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CONFLICT OF INTEREST

The author have declared that no competing interests exist.

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REFERENCES

[1] Alamri R., Ben Abdallah M., Ben Hamza A., Labidi S. Installation d'une unité de production de biofertilisants. Institut National Agronomique de Tunisie. Seminar report 2016, II http://www.memoireonline.com/01/16/9399/.

[2] Faessel L., Tostivin C. Les produits de stimulation en agriculture: un état des connaissances. Notes et Etudes Socio-Economique, 2016, 12 (40), 7-39.

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

[4] Agbodjato A.N., Noumavo P.A., Adjanohoun A., Dagbénonbakin G., Atta M., Rodriguez A.F., de la Noval Pons B.M., Baba-Moussa L. Response of maize (Zea mays. L) crop to biofertilisation with plant growth promoting rhizobacteria and chitosan under field conditions. Journal of Experimental Biology and Agricultural Sciences, 2015, 3(6), 566-574.

[5] Agbodjato A.N., Noumavo P.A., Adjanohoun A., Agbessi L., Baba-Moussa L. 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, Article ID 7830182, 1-11. https://doi.org/10.1155/2016/7830182

[6] Kumar M.NV.R. A review of chitin and chitosan applications. Reactive & Functional Polymers, 2000, 46(1), 1-27.

[7] Sharp R.G. A Review of the Applications of Chitin and Its Derivatives in Agriculture to Modify Plant-Microbial Interactions and Improve Crop Yields. Agronomy, 2013, 3(4), 757-793, doi:10.3390/agronomy3040757.

[8] Deepmala K., Hemantaranjan A., Bharti S., Nishant B.A. A Future Perspective in Crop Protection: Chitosan and its Oligosaccharides. Advances in Plants and Agriculture Research, 2014, 1(1): 6. http://dx.doi.org/10.15406/apar.2014.01.00006.

[9] Hadwiger L.A. CHITOSAN - Molecular Forms with Potential in Agriculture and Medicine. Journal of Drug Design and Research, 2017, 4(2): 1036.

[10] Bornet A. and Teissedre P.L. Intérêt de l'utilisation de chitine, chitosane et de leurs dérivés en œnologie. International Journal of Vine and Wine Sceinces, 2005, 39 (4), 199-207.

[11] Diallo A. and Thiam N. Module de Formation des Formateurs sur les Crabes d'Eau Douce. Wetlands International Afrique: Dakar. 2010, 124 p.

[12] Rurangwa E., van den Berg J., Laleye P.A., van Duijn A.P., Rothuis A. Mission exploratoire « Pêche, Pisciculture et Aquaculture au Bénin » un quick scan du secteur pour des possibilités d'interventions. IMARES Report, 2014, C072/14, 70p.

[13] Jacob S. Valorisation des déchets: La science se penche sur le duo crabe-crevette. Edition N°:5054 Le 29/06/2017, https://www.leconomiste.com.

[14] Kandra P., Challa M.Me, Jyothi H.K. Efficient use of shrimp waste; present and future trends. Applied Microbiology and Biotechnology, 2012, 93, 17-29 https://doi.org/10.1007/s00253-011-3651-2.

[15] Mikpon T., Dah-Nouvlessounon D., Agbodjato N.A., Lehman H., Amogou O., N'tcha C., Mousse W., Sna H., Ahissou H., Adjanohoun A., Baba-Moussa L. Socio-economic and cultural values of two species of crabs (Cardisoma armatum Herklots and Callinectes amnicola Rochebrune) in Southern Benin, Africa: Management of post-harvest losses and exoskeletons. International Journal of Fisheries and Aquaculture. 2020a, 12(2), 36-46.

[16] Mikpon T., Agbodjato N.A., Dah-Nouvlessounon D., Amogou O., Lehman H., N'tcha C., Noumavo P.A., Assogba S., Allagbe M., Ahissou H., Adjanohoun A., Baba-Moussa L. 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. Journal of Global Biosciences. 2020b, 9(10), 2020, pp. 8063-8077.

[17] Aïhou K. Interaction between organic input by Cajanus cajan and inorganic fertilization to maize in the derived savanna of the Benin Republic. PhD Thesis submitted to the University of Abomey-Calavi, Benin, 2003, 182 p.

[18] Adjanohoun A., Noumavo P.A., Sikirou R., Allagbé M., Gotoechan-Hodonou H., Dossa K.K., Yèhouénou B., Glèlè Kakaï R., Baba-Moussa L. Effets des rhizobactéries PGPR sur le rendement et les teneurs en macroéléments

du maïs sur sol ferrallitique non dégradé au Sud-Bénin. International Journal Biological and Chemical Sciences, 2012, 6: 279-288.

[19] Govindappa M., Bharath N., Shruthi H.B., Sadananda T.S., Sharanappa P. Antimicrobial, antioxidant and in vitro anti-inflammatory activity and phytochemical screening of Crotalaria pallida Aiton. African Journal of Pharmacy and Pharmacology 2011, 5(21), 2359-2371. DOI: 10.5897/AJPP11.038

[20] Ruget F., Bonhomme R., Chartier M. Estimation simple de la surface foliaire de plantes de maïs en croissance. Agronomie, 1996, 16(9): 553-562.

[21] Jelin J., Selvakumar T.A., Dhanarajan M.S. Phytological analysis for designinig a microbial consortium to Enhance plant growth. International Journal of Chem. Tech Research, 2013, 5 (3): 1370-1375.

[22] Shaharoona B., Arshad M., Zahir Z.A., Khalid A. Performance of Pseudomonas spp. Containing ACC-deaminase for Improving Growth and Yield of Maize (Zea mays L.) in the Presence of Nitrogenous Fertilizer. Soil Biology and Biochemistry, 2006, 38(9), 2971-2975.

[23] Walker V., Bertrand C., Bellvert F., Moënne-Loccoz Y., Bally R., Comte G. Host plant secondary metabolite profiling shows a complex, strain-dependent response of maize to plant growth-promoting rhizobacteria of the genus Azospirillum. The New Phytologist, 2011, 189(2):494-506. doi: 10.1111/j.1469-8137.2010.03484.x.

[24] Dobbelaere S., Vanderleyden J., Okon Y. Plant Growth-Promoting Effects of Diazotrophs in the Rhizosphere. Critical Reviews in Plant Sciences, 2003, 22(2), 107–149. doi:10.1080/713610853

[25] Cakmakci et al. [] (2006)

[26] Hadwiger L.A., Beckman J.M. Chitosan as a component of pea-Fusarium solani interactions. Plant Physiology, 1989, 66(2), 205–211.

[27] Burd G.I., Dixon D.G., Glick B.R. Plant growth promoting rhizobacteria that decrease heavy metal toxicity in plants. Canadian Journal of Microbiology, 2000, 33, 237-245.

[28] Whipps J.M. Microbial interactions and biocontrol in the rhizosphere. Journal of Experimental Botany, 2001, 52, 487-511.

[29] Orhan E., Esitken A., Ercisli S., Turan M., Sahin F. Effects of plant growth promoting rhizobacteria (PGPR) on yield, growth and nutrient contents in organically growing raspberry. Scientia Horticulturae, 2006, 111(1):38-43.

[30] Wanichpongpan P., Suriyachan K., Chandrkrachang S. (2001). Effects of chitosan on the growth of Gerbera flower plant (Gerbera jamesonii). In: T. Uragami, K. Kurita, T. Fukamizo (Eds.), Chitin and Chitosan in Life Science, Yamaguchi, 2001, 198–201.

[31] Hasegawa A., Kanechika R., Oguni S. Effect of low temperature and chitosan on dormancy breaking and growth of young corms of three Arisaema species. Acta Horticultural 2005, 673: 603-609. doi: http://dx.doi.org/10.17660/ActaHortic.2005.673.83.

[32] Dobbelaere S., Croonenborghs A., Thys A., Ptacek D., Vanderleyden J., Dutto P., Labendera-Gonzalez C., Caballero-Mellado J., Aguirre F., Kapulnik Y., Brener S., Burdman S., Kadouri D., Sarig S., Okon Y. Response of Agronomically important crops to inoculation with Azospirillum. Australian Journal of Plant Physiology, 2001, 28(9): 871–879.

[33] Dobbelaere S., Croonenborghs A., Thys A., Ptacek D., Okon Y., Vanderleyden J. Effect of inoculation with wild type Azospirillum brasilense and A. irakense strains on development and nitrogen uptake of spring wheat and grain maize. Biology and Fertility of Soils, 2002, 36, 284–297.

[34] Lemanceau P., Expert D., Gaymard F., Bakker P.A.H.M., Briat J.F. Role of iron in plant–microbe interactions. Advances in Botanical Research, 2009, 51, 491-549.