

Extraction and Evaluation of Chitosan from Crab Exoskeleton as a Seed Fungicide and Plant Growth Enhancer

^{1,3}Felicity Burrows, ²Clifford Louime, ¹Michael Abazinge, ²Oghenekome Onokpise

¹Florida A and M University, Environmental Sciences Institute, FSH Science Research Center, 1520 South Bronough Street, Tallahassee, FL 32307, USA

²Florida A and M University, College of Engineering Sciences, Technology and Agriculture, Viticulture Center, 6505 Mahan Drive, Tallahassee, FL 32317, USA

³I. M. Systems Group, Inc./National Oceanic and Atmospheric Administration (NOAA), National Centers for Coastal and Ocean Science (NCCOS), 1305 East-West Highway, Silver Spring, MD 20910, Silver Spring, Maryland, USA

Abstract: Proper disposal of seafood wastes is a continuous problem along the Eastern shores of the United States. Blue crab and scallop processing plants continuously dumped their residues into landfills, creating management and environmental concerns associated with ground and drinking water pollution. Additionally, build up of seafood waste generates an unpleasant odor and becomes an eye-sore to both tourists and local communities. Consequently, the present study was designed to evaluate alternate uses for seafood wastes that are economically feasible. The goal of this study was to isolate chitosan from crab exoskeletons and evaluate its potential as a fungicide against seed infection and a plant growth enhancer. Chitosan was obtained by first removing the shell-meat and recovering the calcium carbonate and proteins. Crab exoskeleton samples were demineralized with either 0.5% or 1.0% HCl or 5% or 10% CH₃COOH. A growth enhancement study was conducted with black-eyed peas exposed to the different chitosan treatments. A similar anti-fungal experiment with peanut seeds infested with *Penicillium* was conducted as well. Data on plants height, stem diameter and leaf counts were recorded biweekly for 4 months and analyzed using PROC ANOVA and PROC General Linear Model Statistical Systems. These measurements suggested that seeds pre-treated with the chitosan extracted with 0.5% HCl had the best overall growth and both chitosan (0.5% HCl) and Captan were the most effective in eliminating fungus from peanuts. This research presents plausible possibilities in which seafood waste can be utilized for agricultural purposes.

Key words: Seafood • crab wastes • chitosan • fungicide • plant-growth enhancer

INTRODUCTION

There are many products that utilize organic compounds from seafood waste. Chitin, a natural polymer found in crustaceans is currently being used in numerous medical applications such as bandages to prevent continuous bleeding or as a wound dressing, or to assist in controlling blood cholesterol [1-3]. It is most commonly used as a commercial dietary supplement because of its fat absorbing capabilities [1, 4]. Additional studies conducted show that chitin extracted from crab exoskeleton and its derivatives can be useful in environmental science, specifically for treating waste

water and in agriculture as a plant fertilizer, plant growth enhancer and fungicide [1, 5-7]. The fact that discarded seafood can be utilized in a broad spectrum of health, medical and environmental fields, leads to a better solution for proper disposal of seafood wastes and prevention of excessive build up of seafood waste along the US East coasts.

Crabs are an important seafood product of the United States. The cumulative amount of domestic landings for crabs in both 2003 and 2004, were more than 290,000 metric tons [8]. In addition, more than 70 percent of seafood including crabs is considered processed waste material [9]. In Florida, disposal of seafood wastes is a

continuous environmental concern. Residues from seafood processing plants dumped into landfills have created management and environmental problems related to water pollution [10, 11]. In fact, seafood waste disposal accounts for about 25% of the annual operating budgets of some Florida landfills [12, 13]. Disposal of residues in place other than landfills has produced economic strains for many processing plants, particularly those companies along the eastern shore of the United States (e.g. Florida, Georgia, North and South Carolina shores respectively) that may have already been struggling financially. In addition, build up of wastes tends to attract pests (e.g., flies and mosquitoes), pathogens, encourage bacterial growth, produce offensive odor and create an eyesore to tourists and local communities. The Florida Sea Grant College Program therefore developed a project to help eliminate seafood wastes. To alleviate the stench of the discarded seafood wastes and the burden of already stressed landfills with large amounts of seafood wastes, crab's scraps are mixed with sawdust and bark from trees to produce compost [12, 14, 15]. Other programs and studies have also conducted similar projects using crab-discarded materials and sawdust for various treatments [16-19]. The compost produced can be bagged and bulked in quantity for further use. Granted, discarded waste is utilized as compost, the bulk of seafood waste and its improper disposal is still a problem. This project was therefore designed to evaluate the chemical and physical properties of chitosan isolated from seafood wastes to be used in agriculture and environmental science. This research presented alternative methods of using processed seafood wastes to the scientific community and to government agencies. It also created an awareness of the value and use of organic compounds present in seafood waste. The overall goal of this research was to enhance the utilization of crab waste and help minimize environmental pollution associated with it. The specific objectives for this research were to extract chitosan from crab exoskeletons and 1) evaluate its growth enhancing properties for plants, 2) evaluate its anti-fungal properties for seed storage and preservation.

MATERIALS AND METHODS

Samples preparation: The crabs were obtained from Spears Seafood Market on Lake Bradford, in Tallahassee, Florida. The crab exoskeletons collected were placed in Ziploc bags and refrigerated overnight. Moisture content was determined on the crab waste by first

crushing exoskeletons into smaller pieces using a meat tenderizer. Approximately 10 grams of crushed crab's exoskeletons wet samples were placed on foil paper and measured using a Mettler balance. There were five measurements made of the wet crushed crab exoskeletons samples. The samples were then labeled and oven-dried for 4 consecutive days at 65°C until constant weight. The dry weight of the samples were then determined and the moisture content measured based on the differences between the wet and the dry weight. The average moisture content of the crab exoskeletons was 12.96%.

Extraction of chitin and chitosan: The chitin and chitosan sequence involved the crushing and washing of the discarded exoskeletons as described by Kim [6] and by the Sonat Corporation [20]. The crabs' exoskeletons were placed in 250 ml beakers and treated in boiling sodium hydroxide (2% and 4% v/v) for one hour in order to dissolve the proteins and sugars thus isolating the crude chitin. Since there was little knowledge about what to expect from the 2% and 4% sodium hydroxide (NaOH) concentrations, the criteria established to assess the best results between the two concentrations were simply looking for any visible physical change such as color and/or texture. Based on the fact that both sodium hydroxide concentrations yielded no visual physical change in the crab exoskeleton, the 4% NaOH was selected for use in the chitin preparation, which is the concentration used by the scientists at the Sonat Corporation [20]. After the samples were boiled in the sodium hydroxide, the beakers containing the crab shell samples were removed from the hot plate, placed in the hood and allowed to cool for 30 minutes at room temperature. The exoskeletons were then further crushed to pieces of 0.5-5.0 mm using a Hamilton Beach, 7-speed blender.

Demineralization: The grounded exoskeletons were divided into 4 sub-crab samples weighing approximately 25 g each. Each sub-sample was demineralized with 100 ml of HCl using concentrations 0.5% or 1.0% and the remaining two samples with 5% or 10% acetic acid (CH₃COOH) concentrations. The samples were allowed to soak for 24 h to remove the minerals (mainly calcium carbonate). The demineralized crab shell samples were then treated for one hour with 50 ml of a 2% NaOH solution to decompose the albumen into water soluble amino-acids. The remaining chitin was washed with deionized water, which was then drained off. The chitin was further converted into chitosan by the process of deacetylation.

Deacetylation: The deacetylation process was carried out by adding 100 ml of 50% NaOH to each sample and then boiled at 100°C for 2 h on a hot plate. The samples were then placed under the hood and cooled for 30 min at room temperature. Afterwards the samples were washed continuously with the 50% NaOH and filtered in order to retain the solid matter, which is the chitosan. The prepared chitosan was then placed in 250 ml beakers and labeled according to the treatment used. The samples were then left uncovered and oven dried at 120°C for 24 h. The chitosan was then in a creamy-white form. The moisture percentage of the crab shell samples was then evaluated.

Growth enhancement: Black-eyed peas were selected for this study because it germinates quickly, known to grow well in sandy soil and is a common crop in Florida. They can withstand considerable drought and a moderate amount of shade. Black-eyed peas in Florida reach a canopy height around 20-24 inches. The germination of the seed is rapid at soil temperatures above 18°C. Black-eyed peas have a competitive niche in soils that are sandy [21].

The chitosan powder treatments (Table 1) were applied to black-eyed peas. The black-eyed peas that were treated in this experiment were purchased from Gramling's Incorporated on South Adams Street, Tallahassee, Florida. The chitosan treatments used in this experiment are listed in Table 1. There was also an untreated control for the growth enhancement experiment. The following procedure was used to pre-treat the seeds. Each chitosan treatment was used to treat 5 black-eyed peas in 4 replicates at 3 different time periods: 30 min, 60 min and 120 min. The reason for evaluating time intervals for pre-treatment was to establish whether time exposure to the chitosan powder would have any effect on the biological response of the seeds. The seeds were pre-treated by first shaking the seeds and the powder vigorously for one minute, every 10-minute interval, in a flat glass pan sealed with an air tight rubber cover. This was to ensure even distribution of the chitosan powder on the seeds. The treated seeds and the untreated group (control) were then sown in small plastic trays (3 x 5") containing topsoil. The chitosan treatments were used to treat 20 black-eyed peas and 4 were sown per tray. The trays were then placed outdoors in an enclosed area. Each tray was labeled according to the treatment used on the seeds. Germination and seedling growth was evaluated for 14 days for each treatment.

Chitosan fungicidal action: Chitosan powder treatments and the commercial fungicide Captan were used to treat the peanuts infected with the fungus *Penicillium*. The purpose of this experiment was to test the action of chitosan as a fungicide by eliminating the fungus, over a six-week period compared to a commonly used commercial product. Naturally infected peanuts with the fungus *Penicillium* were provided by Dr. Onokpise, Department of Forestry and Agronomy, College of Engineering Sciences, Technology and Agriculture, Florida A&M University, Tallahassee, Florida. The procedure for conducting this experiment was as follow. The chitosan treatments were used to treat the peanuts. The control used was Captan, a white-powdered commercial fungicide. There was also a non-fungicide treatment to monitor the growth rate of the fungus. A total of 15 peanuts with 5 peanuts per sterilized Petri dish were used for each of the chitosan treatments and control. The fungus equally covered the circumferences of each peanut under investigation. The infected peanuts used, were stored in small tightly sealed plastic containers in a growth chamber (LAB LINE, Model 845) at a temperature of 22°C and relative humidity of 22% until needed for the experiment.

To treat the infected peanuts, they were held with forceps to minimize disturbance of the fungus and then the chitosan powder and Captan were lightly brushed on the seed coat. The treated peanuts were then placed in sterilized petri dishes and maintained in the growth chamber at a 16/8 hour photoperiod for monitoring. The peanuts were evaluated by microscopic observations biweekly for six weeks in order to investigate the rate and success of the chitosan treatments between each other and the Captan in eliminating the fungus. Results were based on inferences made visually by noting the number of peanuts from which the fungus was eliminated when treated with the chitosan treatments and the commercial fungicide Captan. The average and standard error for the amount of peanuts from which the fungus was eliminated for each treatment at the end of the six week study were recorded. This experiment was repeated for another six weeks using 5 infected peanuts per treatment in 3 replicates and data were recorded biweekly. Each time the measurements were recorded, if there was no fungal detection on any of the peanuts it was discarded and recorded.

Field study: The field study was conducted from August to December. For the field studies, 5 black-eyed peas in each of 3 replicates were treated using the chitosan treatments at the best time period (Table 1) established for

germination and seedling growth in the preliminary studies. This was done by first shaking the seeds and the powder vigorously for 30 minutes at 10 minute intervals for 1 min. The powder and the seeds were as described above. The seeds were then stored in Ziploc bags and the treatments were labeled according to the acid concentrations that were used for demineralization for 24 hours at room temperature.

Before the seeds were sown, the soil pH, nitrogen and moisture content were determined in the Environmental Sciences Institute at Florida A&M University, Tallahassee, Florida. The College of Agriculture and Environmental Sciences Laboratory in Athens, Georgia analyzed the nitrogen levels of the soil samples. Field plots were 60" x 60" comprised into rows, each labeled according to chitosan treatments. The treated seeds and the control were then sown in a Randomized Block Design in 3 replicates throughout the experimental area. The seeds were sown 3 feet apart from one another to allow sufficient growth space.

To evaluate chitosan effects on the plant morphogenesis, i.e. average height, stem diameter and leaf number per plant were recorded. The height of the plant was measured as the distance start from the base of the stem near the soil and extended to the apical bud of the plant. The stem base diameter was measured using a 6 inch dial caliper. All these measurements were in centimeters.

The leaves produced for each plant per treatment were counted and then their average numbers per each treatment calculated. Data collection started at the beginning of the second month, in order to allow the plants to fully establish. Measurements were then recorded biweekly within the second month until the end of the fourth month. The measurements were evaluated and analyzed for variations between each of the chitosan treatments and the untreated control. To ensure successful growth of the black-eyed pea plants, the weeds were uprooted biweekly from around the area in which the plant was established, reducing competition from soil nutrients.

Rainfall during the four month study period was recorded biweekly with a Herd Health Pluviometer reading. The average temperatures for each month throughout the four-month study period were recorded. The data for the rainfall during that period was recorded by the employees at the Florida A&M University Research Center in Quincy, Florida. Other observations were noted throughout the experiment such as whether pests attacked the plants causing deterioration or any other biotic and abiotic stress.

At the end of the experiment, soil samples were collected and analyzed for pH, nitrogen and moisture contents. The reason for analyzing the soil's conditions at the end of the experiment was to determine whether chitosan caused any changes in the soil conditions that may have some effect on the growth of the plants. The soil samples were collected approximately 7 inches deep using a soil samples tool. There were 3 soil samples as replicates taken from each treatments area and the untreated control, from points exactly where the plants were grown. The soils samples were then air dried for 3 days and placed in sealed glass jars and labeled according to the area they were collected. For further analysis, the seeds treated with chitosan were then placed in Ziploc bags, labeled according to the area from which they were collected and transported on ice to prevent chemical alterations.

The moisture content of the various soil samples was analyzed and recorded. The pH of the soil was analyzed by first dissolving 30 g of the 3 soil samples from each treatment area, in 100 ml of deionized water. The pH meter then was calibrated and the pH of the soil for each treatment area was then analyzed and recorded. These soils analyses were done in the Environmental Sciences Institute at Florida A&M University, Tallahassee, Florida.

Data analysis: For the growth enhancement and fungicide studies, the data was analyzed using the PROC ANOVA and PROC General Linear Model (GLM) Statistical Analysis Systems (SAS) with a 95% significance level. The differences between the averages were compared using Duncan Multiple Range Test (DMRT). For the growth enhancement experiment, the data were analyzed for the differences between the average percentage germination rates for seeds exposed to chitosan treatments at the various time periods. For the fungicide studies, the data was analyzed and the differences between averages and the standard deviation from which fungus were eliminated on the peanuts for each treatment was indicated. For the field studies, the data was analyzed for the differences between the average leaf count, stem diameter and height for the treatments used and the untreated control. The results that have the same lower case letters indicate that there was no significant difference between them (Table 2).

RESULTS

Samples preparation: The results of the samples preparation are presented in Table 1. The samples

Table 1: Samples preparation and the different treatments used in the study

Crab exoskeleton samples (g)	Wet weight of crab shells (g)	Dry weight of crab shells (g)	Moisture content of crab shells (g)	Acid concentrations used for demineralization	Chitosan treatments
25.2	45.50	26.27	42.10	0.5% HCl	Chitosan _{0.5%HCl}
30.9	66.90	39.10	45.50	1.0% HCl	Chitosan _{1.0%HCl}
25.7	34.90	19.30	44.35	5% CH ₃ COOH	Chitosan _{5%CH₃COOH}
25.1	39.40	24.20	34.90	10%CH ₃ COOH	Chitosan _{10%CH₃COOH}

Table 2: Effects of chitosan treatments on growth of black-eyed peas, fungus-infected peanuts and soil nutrition

Treatments	Seed germination %			Fungal elimination from Peanuts %	Soil moisture content %	pH level	Nitrogen level	Plant morphogenesis		
	exposure time (min)							Mean leaf count	Mean plant height (cm)	Mean stem diameter (cm)
	30*	60	120							
0.5% HCl	90a	60b	80a	**3.7a	14.3	6.1a	0.0222%	***2.88ab	8.81a	0.33a
1% HCl	80a	60b	60b	2.8b	14.9	6.3a	0.0228%	3.29a	7.96bc	0.28b
5% CH ₃ COOH	75a	55b	65b	2.7b	14.0	6.1a	0.0230%	3.04ab	8.28bc	0.26b
10% CH ₃ COOH	85a	55b	40b	2.6b	15.1	6.2a	0.0220%	2.65b	7.25c	0.25b
Control				3.3a	10.8	6.2a	0.0237%	1.80c	5.56d	0.18c
					16.1 (IMC)	6.4a (SP)	0.242% (INL)			

The values listed in the table represent average counts of the replicates taken over the respective growth for the plants or exposure period for the fungicide *Length of time, the seeds were exposed to the chitosan. **Average number of peanuts from which the fungus was eliminated after exposure to chitosan. ***Within a column, data with the same letter are not significantly different at the 5% level (P<0.05)
 IMC (Initial Moisture Content of the soil), SP (Starting pH of the soil at the beginning of the experiment), INL (Initial Nitrogen Level of the soil at the beginning of the study)

preparation included extraction of chitin and chitosan, demineralization and deacetylation of crab exoskeletons. For each treatment used (Table 1), the amount of crab exoskeleton collected varied from 25 to 30 g, while the wet weight of crab ranged from 34 to 67 g. The samples preparation included the dry weight and the moisture content of the crab samples as well, which ranged from 19 to 39 g and 34 to 45 g, respectively.

Growth enhancement: The results for the laboratory studies enhancement experiment are shown in Table 2. The data represent the percentage seed germination after being treated for the various time periods. Results showed that the seeds exposed to chitosan for 30 min prior to planting achieved a better germination percentage when compared to those exposed to chitosan for 60 and 120 min respectively. Those exposed for 60 min showed less germination than those treated for 120 min, however there was no significant difference between these results. The seeds exposed for 30 min to chitosan showed higher germination rates (%) compare to the untreated control. Also, the seeds exposed to chitosan treatments were taller than the untreated control. Furthermore, the seeds

treated with chitosan 0.5% HCl for 30 min, produced the greatest percentage of germination compared to the other treatments. However, the germination ratio (%) for plants exposed to chitosan 0.5% HCl was homogenous with the one of the untreated control.

Chitosan fungicidal action: The results of the laboratory studies for the fungicide experiment at the end of the 6-week period are shown in Table 2. The total number of peanuts that were exposed to each treatment was 15. Within the first two weeks, none of the treatments were effective in reducing or eliminating the fungus, because *Penicillium* was still present on all of the peanuts. At the end of the fourth week, chitosan 0.5% HCl, chitosan 1.0% HCl and chitosan 5% CH₃COOH treatments eliminated fungus from 53% of the peanuts. The chitosan 10% CH₃COOH treatment and control eliminated 47% of the fungus.

At the end of the six-week period, chitosan 0.5% HCl eliminated fungus from 73% of the peanuts; chitosan 1% HCl eliminated fungus from 60% of the peanuts; chitosan 5% CH₃COOH eliminated fungus from 57% of the peanuts and the control eliminated fungus from 67% of the

peanuts. From these studies, chitosan 0.5% HCl and the control showed the best response as anti-fungal agents under laboratory conditions. There was no significant difference ($P < 0.05$) between the chitosan 0.5% and the control.

Field study: The results of the field studies shown in Table 2, presented average leaf count, height and stem diameter of the plants that were under investigation for the four month study. The results for average leaf count of plants whose seeds were treated with chitosan 1.0% HCl and chitosan 5% CH_3COOH treatments, revealed better leaf production. The average number of leaves when treated with chitosan 1% HCl and chitosan 5% CH_3COOH were 3.29 and 3.04 respectively. The average leaf count for the untreated control was 1.80 therefore were less than the plants whose seeds were treated with chitosan treatments. The average height of the plants treated with chitosan was greater than the untreated control. However the height of the plants treated with chitosan 0.5% HCl and chitosan 5% CH_3COOH was better than all other plants. The average height for chitosan 0.5% HCl and chitosan 5% CH_3COOH treatments were 8.81 and 8.28 cm respectively. The overall results for the untreated control in regard to the height of the plant were significantly lower than the experimental plants, therefore the height growth process was much slower for the controls.

For evaluations of the stem diameter, plants in which the seeds were treated with chitosan 0.5% HCl gave the best results indicating that the plants readily established themselves. The average stems diameter of the plants when treated with chitosan 0.5% HCl was 0.32 cm. For the untreated control, the average stem diameter was 0.18 cm. There were also some pest and predator activities during the study which might have affected the growth success of the plants. Pests such as beetles, caterpillars and butterflies attacked the plants deteriorating the quality of the leaves of the plants. Also deer trampled through the experimental area disturbing the growth process of some of the plants. However all plants were exposed to the same conditions at all times.

DISCUSSION

According to the results of the growth enhancement experiment conducted in the field, it appears that the chitosan treatments were successful in giving a better plant growth than the untreated control. Chitosan, which is chemically an amino sugar, β -D-glucosamine, may have

assisted in stimulating the synthesis of protective agents [7]. Chitosan and its oligosaccharides contributed to plant growth by acting as natural elicitors or Catalysts inducing pathogenesis related proteins such as chitinase enzymes for young seedlings. As indicated by Ohta *et al.*, [7], chitinase enzyme is already present in the plant as a defense mechanism but with the assistance of chitosan the defense mechanism is stimulated earlier than the normal cycle of the seedlings growth therefore providing protection at an earlier stage.

In black-eyed peas, chitosan may have acted as a signal for cellulose response of the chitinase enzyme in the plants to initiate their defense mechanism against phytopathogenic infections. Once the defense mechanism of the plants was initiated by the chitosan and chitinase enzyme activities, the plants were able to grow more productively without undergoing any deterioration due to infection. Chitosan inducing the defense mechanism of the plants also provides protection against environmental stress such as drought and maintains stability of the plant. For instance, the stems of the chitosan treated plants were much thicker than the untreated control. As the temperature decreased from August to December the stems of the treated plants continued to thicken. The stems of the untreated control however were much thinner during the dry season, which was between November and December.

A previous study was done on the effect of chitosan treatments on plants during heat stress by Duke and Doehlet [22]. The results of their study were consistent with the idea that increased heat affected the productivity of plants. However with decrease in temperature, producing cold stress during the growth enhancement study, chitosan was still able to maintain healthy growth. This then presents the idea that if modifications are made to the production and application of chitosan, the plants should be able to grow productively under heat stress.

Since the nitrogen levels in the soil samples for areas where chitosan treatments on plants were used were lower than the soil samples of the control, this indicated that chitosan may have induced the absorption capacity of the treated plants (Table 2). Therefore nitrogen was readily taken up by chitosan treated plants contributing to their successful growth. Also chitosan alone contains approximately 6.8% of all other minerals present in the crab shells, therefore the additional nitrogen was utilized by the chitosan treated plants [23].

The β -D-glucosamine, chitosan also contributed to the absorption of water present in the soil. The more moisture in the soil, the more β -D-glucosamine becomes

present increasing the plants absorption ability. Chitosan also assisted in conserving water in the plants by closing the stomata and decreasing transpiration and maintaining production of plants [24]. As a result, during the dry season when rainfall was low, the chitosan treated plants were able to provide themselves with water to prevent wilting and continued to circulate the nutrients in the plant for successful growth. Therefore the numbers of leaf foliage for chitosan treated plants in this study was greater than the untreated control due to the ability of these plants to conserve more water. The untreated control during the dry season was not able to provide itself with additional water, so began to deteriorate as the season changed. At the end of the experiment the pH level of the soil for the various sample areas including the untreated control slightly decreased from the initial pH of the soil. The pH of the soil was between 6.0 and 6.5 (Table 2). The change in pH of the soil was not significant, therefore had no tremendous impact on the growth of the plants.

Though the chitosan treatments were more successful in growth compared to the untreated control, there were some variations in the results among the chitosan treated plants. For instance the plants treated with chitosan 0.5% HCL gave the best overall result in the growth enhancement study because it had the best results for 2 of the 3 characteristics that were under investigation. The characteristics included achieving a greater height and stem diameter. The chitosan 1.0% HCL treatment achieved a greater percentage for leaf production. Although plants treated with chitosan 5% CH₃COOH did not give the best overall results. It had a relatively good performance in terms of percentage for average leaf count and height of the plants.

The 0.5% HCL acid that is commonly used by scientists in the demineralization process [25] and used in this experiment for chitosan production, can therefore be substituted with a weak, organic and more environmentally friendly acid such as the acetic acid used in the demineralization process. Since chitosan 5% CH₃COOH produce significant good results in the growth enhancement study, it therefore has the potential to successfully be applied to other environmental and agricultural areas once further modifications are made to the methodology to enhance chitosan production. The results for chitosan 10% CH₃COOH treatment after evaluating the success of the plants average height, leaf count and stem diameter were significantly lower than the results of the other chitosan treatments. This indicated that a higher percentage of acetic acid used did not

produce beneficial results. However the results for plants treated with chitosan 5% CH₃COOH were more successful than the results of the untreated control. The results for this study were compatible with the growth enhancement results done by Goosen [26].

As for the effects of chitosan and Captan treatments on fungal growth, chitosan 0.5% HCL treatment gave the best result in eliminating and inhibiting fungal growth from the peanuts for both the preliminary and final study. Chitin is a protein that is present in the fungal cell walls, assisting with cell structure. However chitosan hydrolyzes chitin in the cell walls and deteriorate the cell structural component, such as cell wall thickening and hyphal distortion [27]. Chitosan has good adherence capabilities. Once applied to the seed coat of the infected peanuts, it immediately adhered to the fungus and began its reaction. Chitosan acted as a catalyst stimulating chitinase enzymes, initiating the defense mechanism of the peanut. Once the defense mechanism was initiated, the fungal cell walls underwent some disturbances. The chitosan began to destroy the cell walls by causing leakage of amino acids and proteins which support the fungal cell walls causing cytological damage [5]. As a result the cell walls began to erode. Therefore chitosan 0.5% HCL treatment was consistent with its effectiveness for plant growth enhancement as well as a fungicide.

However, the commercial product captan was more effective than chitosan 1% HCL, chitosan 5% CH₃COOH and chitosan 10% CH₃COOH treatments in inhibiting and eliminating the fungal growth of *Penicillium*. Research conducted previous to this study, indicated that the commercial fungicide prochloraz was more effective in eliminating *Penicillium* than the chitosan treatments under investigation [28]. However the Chitosan 0.5% HCL treatment gave better results for fungal elimination than the commercial fungicide captan. Of course the prochloraz may be a more effective fungicide than captan, but its effectiveness compared to chitosan treatments in this study is yet to be investigated. Therefore further investigations can be conducted to evaluate fungal elimination and inhibitory activity of the chitosan 0.5% HCL treatments compared to other commercial fungicides that are being used today.

There was one difference in the results between the laboratory studies of the fungicide experiment and the final fungicide experiment. The number of peanuts in which the fungus was eliminated was greater for the laboratory studies than the final experiment. This is because the fungal growth on the peanuts for the laboratory studies was less abundant on the

circumference compared to the peanuts in the final experiment. As a result, the chitosan treatments and captan were able to eliminate the fungal growth more readily. However from simple microscopic observations, the fungus on the peanuts in the final fungicide experiment did undergo some reduction on the circumference of the peanuts that were treated compared to the initial abundance of fungus that was present. This indicated that given a longer time of exposure to treatments, eventually the fungal growth of the *Penicillium* would have been significantly reduced.

CONCLUSIONS

Improper disposal of seafood waste materials has become a serious environmental concern for coastal areas. This research introduces plausible possibilities in which seafood waste can be utilized for agricultural purposes. The use of natural waste products within the environment can not only eliminate build up but also reduce excessive use of chemicals in the environment that may result in terrestrial and aquatic pollution. From the results of the present research specifically, the growth enhancement experiment, the chitosan was successful in increasing the growth of plants and can be a potential fertilizer. Though its fertilizing capability was not compared to any organic and inorganic commercial fertilizer that are being used, it still presented the possibility of providing similar results when compared to commercial fertilizers. Chitosan when treated on the seeds, has the potential to inhibit microbial and pathogenic activity. Therefore the amount of commercial pesticides that are used and enter into our water sources through runoff may be reduced. Chitosan entering our water sources may not cause adverse effects on the environment, since it is a compound found in nature and only small amounts are used. Chitosan can then be evaluated as a pesticide. Other considerations when using chitosan should be evaluated under various abiotic conditions such as change in temperature, pH and salinity levels. We can increase or evaluate the effectiveness of chitosan on plant growth during changes in seasons, in various types of environments such as tropical or coastal inlands and whether these abiotic factors may also contribute to promoting chitinase enzymes increasing plant growth.

Since chitosan provides a protective layer against bacteria and fungi that may result in decay, it may be considered a good source for preservation of seeds and fruits during storage. The chitosan preservative ability then can be compared to commercial preservative

that are being used today. The use of chitosan and other processed seafood waste material for agricultural purposes, instead of some commercial fertilizers and fungicides that are being used today, can prevent excess amounts of chemical buildup that may enter the environment resulting in pollution.

ACKNOWLEDGEMENTS

We sincerely thank Dr. Elijah Johnson, Dr. Jennifer Cherrier for their insightful comments and guidance during the course of this project and Jeff Ino Baptiste for much needed field assistance. This work was financially supported by the Graduate School, the Environmental Sciences Institute, the College of Engineering Sciences, Technology and Agriculture, the Quincy Research Farm and an initial grant from USDA-AS-OICD-RESD.

REFERENCES

1. Goosen, M., 1996. Applications of Chitin and Chitosan, CRC Press, USA.
2. Simontacchi, C.N., 1999. All about chitosan, (Faqs all about health series), Mass Market Paperback.
3. Spiller, G.A., 2001. CRC, Handbook of dietary fiber in human nutrition
4. Hennen, W., 1999. Chitosan: Natural fat blocker, Woodland Publishing Incorporated.
5. El Ghaouth, A., J. Arul, J. Grenier and A. Asselin, 1992. Effect of chitosan on cucumber plants: Suppression of *Pythium aphanidematum* and induction of defense reactions. *Phytopathology*, 84: 313-320.
6. Kim, D., 1999. Dalwoo - Chitosan Corporation, Chitin, chitosan and chitosan bloomer: preparation of chitin and chitosan. www.members.tripod.com/~Dalwoo.
7. Ohta, K.T., Asao and T. Hosoki, 2001. Effects of chitosan treatments on seedling growth, chitinase activity and flower quality in *Eustoma grandiflorum* (raf.) Shinn 'Kairyuu Wakamurasaki. *J. Hort. & Biotechnol.*, 76: 612-614.
8. NOAA Fisheries, 2004. Fisheries of the United States 2004. US Commercial Fishery Landings, Species. http://www.st.nmfs.gov/st1/fus/fus04/02_commercial2004.pdf
9. Brown, K.F., 1981. Crab meal production: tragic impact on the blue crab industry unless viable alternatives established, Conference on Seafood Waste Management in the 1980's; Orlando, FL (USA); September 23-25, 1980. Florida Sea Grant College; Gainesville, FL (USA), pp: 280-284.

