escola superior de turismo
e tecnologia do mar
instituto politécnico de leiria

Chitosan as seed soaking agent: germination and growth of Coriandrum sativum and Solanum lycopersicum

Gonçalo Alexandre Teixeira de Castro

2017

Chitosan as seed soaking agent: germination and growth of Coriandrum sativum and Solanum lycopersicum

Gonçalo Alexandre Teixeira de Castro

Project to obtain the Master Degree in Marine Resources Biotechnology

Project conducted under the guidance of Prof. Dr. Sérgio Miguel Franco Martins Leandro
(Escola Superior de Turismo e Tecnologia do Mar – IPL, Portugal).

2017

Title: *Chitosan as seed soaking agent: germination and growth of Coriandrum sativum and Solanum lycopersicum.*

Acknowledgements

Quero agradecer em primeiro lugar a toda a minha família, namorada, Sr. António Eugénio e D. Natalina que me apoiaram, se esforçaram e lutaram sempre ao meu lado em todas as minhas batalhas, limpando-me as lágrimas ao longo destes anos para que eu finalmente chegasse a este momento.

Gostava de agradecer ao meu grande amigo Francisco Avelelas por me ter aconselhado durante estes quase três anos de forma incansável. Ajudou-me em todas as minhas dificuldades sem nunca pedir nada em troca, nunca me vou esquecer do grande apoio que ele me deu.

Agradecer a professora Susana Mendes por me ter ajudado na análise estatística, foi também sempre espetacular e sempre predisposta a ajudar-me nas dúvidas.

Agradecer a todos os meus amigos, em especial ao João Reboleira, que me ajudou e sempre me apoiou diariamente. Queria também agradecer a todas as pessoas que estiveram nos momentos mais difíceis, na avaliação da minha tese e os que tiveram menos presentes, mas sempre que podiam ajudavam-me de alguma forma.

Por último agradecer ao professor Sérgio Leandro por me ter aceite e dar a oportunidade de fazer a tese com ele.

Agradecer também a todos os meus professores por me darem os ensinamentos necessários para que pudesse ser alguém e vingar na vida.

Resumo

A crescente exigência mundial por alimentos representa uma preocupação cada vez maior para com os recursos agrícolas. De modo a atender a exigência resultante de estudos demográficos, é extremamente importante melhorar as tecnologias de produção de modo a alcançar uma elevada produção agrícola. Fertilização apropriada e eficiência de controlo de pestes, em culturas, é essencial para se obter produções de alta qualidade.

Para melhorar a resistência de culturas contra oscilações de temperatura e doenças, várias estratégias foram testadas, como o revestimento de sementes (Omolehin et al., 2007). Embora as práticas atuais aumentem efetivamente a germinação e reduzam as perdas, os agentes convencionais de revestimento de sementes não são a melhor alternativa, pois dependem da adição de fertilizantes químicos e pesticidas no solo, levando a custos ambientais. Além disso, as pragas podem desenvolver resistência contra estes tratamentos, o que implica o uso de quantidades cada vez maiores de produtos químicos para manterem a eficiencia (Altieri e Nicholls, 2003). Portanto, há uma exigência crescente por alternativas mais seguras e mais ecológicas.

O quitosano é um polissacárideo catiónico produzido pela *N-desacetilação* alcalina da quitina. A carga positiva do quitosano confere inúmeras e únicas propriedades fisiológicas e biológicas com grande potencial em uma ampla gama de indústrias, como tratamento de água, processamento de alimentos, medicamentos e farmacologia (Rinaudo, 2006). Além disso, o quitosano foi reconhecido como um produto com a capacidade de aumentar a produção de culturas devido às suas bio-atividades: biodegradabilidade, estimulação de crescimento e germinação de sementes, aumento da absorção de nutrientes, redução do stresse oxidativo, aumento do teor de clorofila, alargamento fotossintético e cloroplasto nas folhas, antifúngico, antiviral e propriedades antibacterianas (El Hadrami et al., 2010; Hadwiger, 2013). Portanto, este pode ser usado como pulverização foliar, revestimento de sementes, mergulho em raiz de plântulas; enriquecimento do solo ou como suplemento em meios de tecidos vegetais (Pichyangkura, R. e Chadchawan, 2015).

Este recurso marinho (subproduto processamento pescado) e as suas propriedades biológicas, apoiam a hipótese de usá-lo como matéria-prima para as indústrias

biotecnológicas, aumentando dessa forma seu valor economico. A única desvantagem foi a concentração utilizada (quitosano a 1%), sendo esta concentração inibitoria para o desenvolvimento das espécies testadas.

Nesse contexto, o objetivo foi avaliar o efeito do quitosano extraído de recursos marinhos ao nível da germinação e desenvolvimento das espécies *Solanum lycopersicum* (tomate) e *Coriandrum sativum* (coentros).

Palavras-chave: Quitosano; imersão sementes; *Solanum lycopersicum*; *Coriandrum sativum*; germinação

Abstract

The world´s growing demand for food poses major concern on agriculture resources. In order to meet the demand resulting from demographic studies, it is extremely important to improve the production technologies in order to achieve high agricultural productivity. Proper fertilization and efficient control of pests in cultures are required to obtain a high quality production.

In order to improve crop resistance against temperature fluctuations and diseases, several strategies have been tested, such as seed coating (Omolehin et al., 2007). Although current practices effectively enhance germination and reduce losses, the conventional seed coating agents are not the best alternative, relying on the addition of chemical fertilizers and pesticides to the soil, leading to environmental costs. In addition, pests may develop resistance against them, which implies the use of increasingly larger amounts to be efficient (Altieri and Nicholls, 2003). Therefore, there is an increasing demand for safer and more eco-friendly alternatives.

Chitosan is a cationic polysaccharide produced by the alkaline N-deacetylation of chitin. The positive charge of chitosan confers numerous and unique physiological and biological properties with great potential in a wide range of industries such as water treatment, food processing, medicine, and pharmacology (Rinaudo, 2006). Furthermore, chitosan have been recognized as a product to enhance crop production due to its bioactivities: biodegradability, growth stimulation and seed germination, increasing nutrient uptake, reducing oxidative stress, increasing in chlorophyll content, photosynthetic and chloroplast enlargement in the leaves, antifungal, antiviral and antibacterial properties (El Hadrami et al., 2010; Hadwiger, 2013). Therefore, it can be used as foliar spraying, seed coating, seedling root dipping; soil enrichment or as supplement into plant tissue media (Pichyangkura, R. and Chadchawan, 2015).

This marine resource (by-product processed fish) and its biological properties support the hypothesis of using it as a raw material for the biotechnology industries, thereby increasing its economic value. The only disadvantage was the concentration used (chitosan at 1%), being inhibitory for the development of the tested species.

In this context, the objective was to evaluate the effect of chitosan extracted from marine resources on the germination and development of *Solanum lycopersicum* (tomato) and *Coriandrum sativum* (coriander) species.

Key-words: Chitosan; seed soaking; *Solanum lycopersicum*; *Coriandrum sativum*; germination.

Index

Figure index

Table index

Chapter 1: General introduction

1.1. Crop enhancement – new trends

Crops provide human important products to ensure the security of food, being one of the key's global challenges of this century. With the increase of the global population, climate changes and the overuse of toxic compounds that degrade the fertile soils, great challenges are currently been faced towards higher quantity and quality of products to feed the world (Davies et al., 2009; Hawkesworth et al., 2010). Multiple research areas of crop science and technology, including genetics, agronomy, germplasm resources, crop management practices and crop biotechnology, try to stimulate innovation towards a more sustainable crop production, ensuring his way global food security.

Plants are essential to sustain for world feeding habits, due to their content in secondary metabolites and nutrients such as phenolic compounds, terpenes and alkaloids, related with various biological processes such as seed dispersal and resistance to stresses (Ruhul Amin et al., 2009; Jahangir et al., 2009). Intensive practices focus on the use of improved crops, mechanical plowing, chemical fertilizers and pesticides. However, they pose a negative environmental impact, raising doubts if this is the right way to face current challenges. Organic agriculture free of pesticides is considered environmental friendly, producing healthier foods due to better nutrients composition, although scientist raise concerns about its capacity to feed a growing population, its management efficiency of plant pests and diseases, as well as managing potential adverse impacts (Azadi et al., 2011).

Improvements in agriculture have usually focused on providing more "confort" to plants to increase yield (Bennett et al., 2012). However, these measures have depressed the synthesis of phytochemicals since these are usually produced during stressful conditions such as drought, irradiation, heat and salinity (Frost et al., 2008). Phytochemicals are needed by plants to defend themselves in a hostile environment and are useful to consumers, bringing beneficial effects on health (Rea et al., 2010). For that, improvements in agricultural practices should focus not only on yield, but also on the maintenance of phytochemicals present in plants.

1.2. Factors that affect crops

Seeds are specially vulnerable to stress during seedling establishment (Carter and Chesson 1996), which ultimately affects all their life cycle. Osmotic stress generally results in hormonal imbalance as well as in the reduction of leaf chlorosis, antioxidants and plant growth (Khan 1992; Flowers 2004; Ashraf et al. 2010). The presence of abiotic stress caused eitherby climatic and/or environmental conditions, influences crops optimal growth due to changes in temperature, pH and extreme humidity or severe drought, etc (Lafitte, 2001; Boyer and Westgate, 2004; Tester and Bacic, 2005).

Also, excessive water may cause plant rotting , by forming a sludge that stores water for several days without renewing, resulting in optimum conditions for opportunistic microorganisms which colonize the plant roots, consuming important nutrients for plants development, reducing plant growth and resulting in irreversible damages (Widstrom, 1996; Placinta et al., 1999).

In addition to watering conditions, the pH is used as a key parameter in the evaluation of soil and water. If the soil is too acidic, some metal ions become toxic, while with a slightly alkaline pH, problems of low solubility of ions or high levels of sodium and metal sulfides can be found (Magnavaca and Bahia Filho, 1993; Aguirre, 2001).

Drought is one of the most important negative impact in global agriculture production, affecting both vegetative and reproductive moments of crops (Talwar et al. 2002). Climatic changes are also expected to have substantial impacts on the intensity and frequency of future droughts (Manabe et al. 2004; Harte et al. 2006). The excessive energy trapped in the chloroplasts, but not used forcarbon fixation, can result in potentially harmful conditions for chloroplasts structures, due to over-reduction of the photosynthetic electron transport chain, resulting in theproduction of reactive oxygen species (ROS; Reddy et al. 2004).

1.3. Chemical fertilizers

The chemical fertilizers played an important role in enhancing crop production, been reported as effective and economically feasible among the smallholder farming sector where conservation agriculture (CA) is being practiced (Muoni et al., 2013). However, overuse of fertilizers resulted in a reduction of water quality and greenhouse gases (GHG) emissions causing environmental damages (Ju et al., 2006, 2009; Cui et al., 2013), and posing potential toxic side effects in humans (Kolpin et al., 1998).

They are the most common and effective approach to control the crops enhancement, by killing, weakening or suppressing weeds (herbicides). A variety of chemicals are available depending upon their mode of action, chemical composition, formulation, selectiveness and efficacy. The most common chemicals used are glyphosate, paraquat, glufosinate, 2,4-D and dicamba (Vargas et al., 2005), with negative effects previously reported in several publications.

1.4. Sustainable approach

The reduction of pesticides to control both, pests and pathogens and the presence of high amount of nutrients in food, is as important as food production. Increasing yields on existing agricultural land is considered the key element for minimizing further expansion. For that, farmers and scientists rely on plants defensive mechanisms, suitable to develop a more sustainable production, providing not only improvement in consumer's health but minimize the use of pesticides used (Wirsenius et al., 2010; Capanoglu, 2010).

Elicitors (Fig. 1) have a huge role in plant defense mechanisms (Holopainen et al., 2009; Montesano et al., 2003). In fact, it is known that treatment of plants with elicitors, or attack with pathogens, causes a set of chain reactions such as the accumulation of defensive secondary metabolites in edible and inedible parts of plants (Kim et al., 2006; Ferrari, 2010; Capanoglu, 2010; Saw et al., 2010).The use of elicitors in organic agriculture, as well as integrated agricultural systems, can contribute to sustainable food production systems. Due to the lower yield of organic agriculture, the use of elicitors could become a viable strategy to the sustainability and development of agricultural systems (Mejía-

Teniente et al., 2010). The right elicitor, in the right concentration, when applied to crops, reduces the use of agrochemicals and in addition, the yield achieved with agriculture practices or conventional practices can be maintained or improved.

Elicitors can be classified according to their biological origin as biotic (polysaccharides, microorganisms, glycoproteins) or abiotic (temperature, fungicides, antibiotics, heavy metals, pH stress) (Walters et al., 2005; Ramakrishna and Ravishankar, 2011). Some examples are jasmonates, salicylic acid (SA), benzothiadiazole (BTH), Etephon, hydrogen peroxide, and oligosaccharides such as chitosan (Jeong and Park, 2005; Bautista-Baños et al., 2003). The use of elictors obtained from wastes has attracted considerable attention.

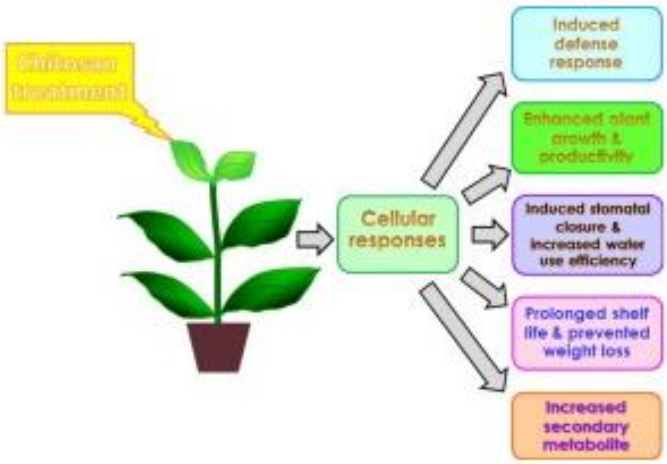


Figure 1 – Chitosan as biostimulant in horticulture (pichyangkura and Chadchawan, 2015)

1.5. Marine resources for a sustainable growth

The marine environment continues to be a source of unique natural products used for pharmaceutical and biotechnological applications (Houssen W. and Jaspars M., 2012).

In recent years the sub-products discarded and the products resulted from fish processing industries, have received much attention due its economic and environmental impacts (Ferrari et al., 2010).

These discarded crustaceans, are source of several biocompounds of great importance for biotechnological companies. Biopolymers, such as chitin are present in the exoskeletons of these discarded organisms, which can be used for the development of high-value products such as chitosan.

Chitosan is a biopolymer of glucosamine and N-acetylglucosamine residues. This biopolymer is therefore produced from chitin, mostly from crab shells, shrimp shells, squid pens and, in some cases, from fungi, via a heterogeneous deacetylation process (Kumaresapillai et al., 2011; Muñoz et al.,2015; Nwe et al., 2011), where solid chitin is soaked in 40–50%(w/v) NaOH. Deacetylation of chitin normally remove over than 80% of the acetyl groups from de N-acetyl-d-glucosamine residues, converting it into D-glucosamine, to yield chitosan, normally referred as percentage degree of deacetylation (DD). The deacetylated product can be solubilized in weak organic acids such as ascorbic acid, acetic acid or lactic acid.

The starting material derived from chitosan can be quite different in terms of the average molecular mass, molecular mass dispersity and DD. These parameters can greatly affect the physical properties and biological functions of chitosan, such as the solubility and the ability to stimulate plants. Therefore, these parameters should be well defined for certain application. The differences in the starting material can be reduced or eliminated by NaOH pre-treatment to modulate DD, polymer size and polydisperisity (Jung and Park, 2014; Kubota et al., 2000; Thadathil and Velappan, 2014).

1.6. Adjuvants

Adjuvants are used to increase the efficiency of the product or modify some properties of solutions, aiming to facilitate the application or minimize possible impacts to the environment. They are commonly added to the herbicide spray solution for enhance

performance, improving weed control efficiency (Hartzler and Foy, 1983; Green et al., 1992; Bunting et al., 2004).

The adjuvants are divided in two groups; (i) liquid superficial properties modifiers and (ii) additives or non-ionic surfactants and fertilizer salts (NISs). Adjuvants that have properties to modify the superficies of liquids are call surfactants and the additives are for example mineral oils or vegetable, urea ammonium nitrate (UAN), ammonium sulfate (AMS) (Monaco et al., 2002), among others. These additives are generally used in combination with a surfactant or another adjuvant to improve herbicide efficacy on weed species (Nalewaja et al., 1995; Bunting et al., 2004; Dodds et al., 2007; Pearson et al., 2008).

Adjuvants are commonly used to modify the physical and biological properties of spray mixtures to improve its chemical performance (Kudsk and Mathiassen, 2007; Zhu et al., 1997). They also influence spray atomization and formation, which is important because each type of application requires a certain optimum droplet size for its biological activity. Different plant species have different farming protocols taking into account their spray deposition, uptake, retention, translocation and biological activity on plants surface (Hunsche et al., 2006; Kraemer et al., 2009; Spanoghe et al., 2007).

Amer et al. (1993) reported a 30% increase in control of powdery mildew of wheat, when adjuvants were mixed with fungicides or pesticides. Tank-mix adjuvants are used worldwide in order to improve the efficiency of foliage applied pesticide formulations, especially if these are to be used at reduced dose rates. A wide range of additives have been shown an improvement spray deposition on foliage, notably surfactants, emulsificable oils and polymers (de Rutter et al., 1990; Holloway, 1994; Nalewaja et al., 1996; Hoyle et al., 1998; Hall et al., 1997a,b, 1998; Wirth et al., 1991; Richards et al., 1998).

Of particular interest are polymers, as these are becoming more widely used as formulation components for active ingredients (Narayanan and Chaudhuri, 1992). Film forming polymers are widely used as spray adjuvants within the agricultural, forestry and horticultural industries (Backman, 1978). The main functions are to reduce pesticide use, act as sticker to improve distribution and adherence of agrochemicals, decrease water loss and wilting of young transplants (Gale and Hagan, 1966).

Previously published studies showed that film forming polymers could be an alternative to fungicides, given its effectiveness controlling foliar pathogens of cereals, vegetables, fruit and ornamentals (Blaedow et al., 2006; Sutherland and Walters, 2001, 2002), preventing adherence of spores that subsequently inhibit germ tube development (Han, 1990 ; Osswald et al., 1984), are less phytotoxic than synthetic fungicides to leaf tissue, permeable to atmospheric gases and allow penetration of solar radiation (Fuller et al., 2003). Since film forming polymers act by physical and not by chemical means, they are not subject to the restrictions of legislation (Anon., 2008) and consequently offer a cheaper (polymers cost 40–80% less than conventional fungicides) and environmental friendly system.

1.7. Chitosan and its derivatives sustainable for agriculture as plant elicitors

Chitosan and their derivatives (Fig. 2) are biorenewable, non-toxic, environmental friendly, biocompatible, biodegradable and biofunctional (Zargar et al., 2015). In addition, it also has a great potential when applied in agriculture, enhancing crop production (Chibu and Shibayama, 2000; Wongroung et al., 2002; Chmielewski et al., 2007; Dzung and Thang, 2002, 2004; Dzung, 2005, 2007, 2010; Dzung and Thuoc, 2006; Bukrudeen et al., 2010). Moreover, this biopolymer also presents antibacterial and antifungal activities, being used as biofungicide for plants, and at the same time, enhancing quality of agricultural products (Darvill et al., 1992; Dzung et al., 2006; Dzung and Thuoc, 2006; Guo et al., 2008, Rinaudo, 2006; Yin and Du, 2010; El Hadrami et al., 2010; Ramos-Garcia et al., 2009; Wongroung et al., 2002; Li and Tao, 2009).

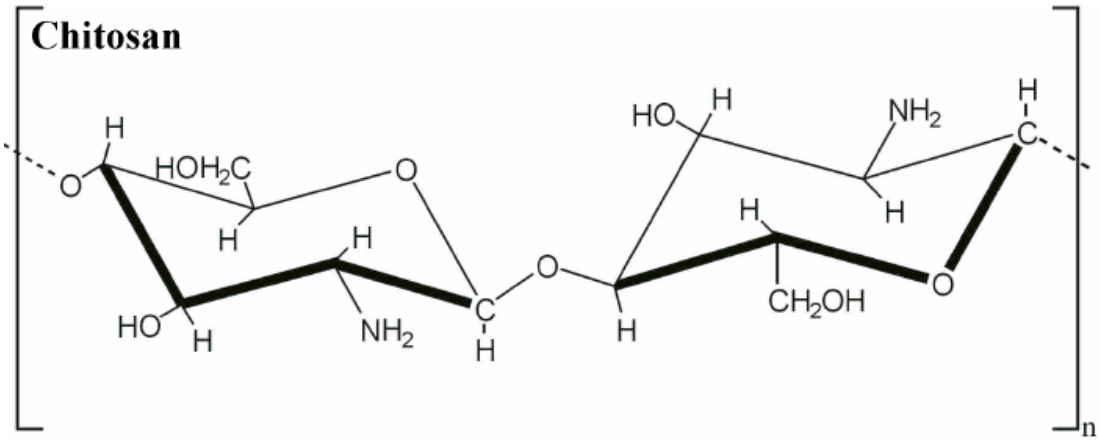


Figure 2 - Chemical structure of chitosan. (López-García et al., 2014).

Chitosan applied as plant elicitor can stimulate plant growth and seed germination, increase chlorophyll content, photosynthetic and chloroplast enlargement in the leaves, increase of nitrogen fixation, nutrient uptake and stress reduction (Chandrkrachang, 2002; Li and Wu, 1998; Luan et al., 2006, 2005; Nge et al., 2006; Reddy et al.,1999; Sharathchandra et al., 2004; Limpanavech et al.,2008; Dzung and Thang,2002, 2004; Lerouge et al.,1990; Dzung, 2005, 2007, 2010, Dzung et al., 2011; Bittelli et al., 2001; Chibu and Shibayama, 2000; Wongroung et al., 2002; Chmielewski et al., 2007; Dzung and Thuoc, 2006; Bukrudeen et al., 2010).

Due to its significant properties, the application of chitosan and its derivatives has been studied in many crop species, including cereal, ornamental, fruit and medicinal crops (Chandrkrachang, 2002; Chibu & Shibayama, 2000; Chmielewski et al., 2007; Dzung and Thang, 2002, 2004; Dzung, 2005, 2007; Dzung & Thuoc, 2006; Dzung et al., 2006; Li and Wu, 1998).

Secondary metabolites have been confirmed to possess many bioactive properties, activated by plant defences systems, would/could reduce the use of pesticides. Chitosan, methyl jasmonate and salicylic acid, among others elicitors, have been reported to be able to mimic biotic and abiotic stresses such as wounding, pathogen attack, UV light exposure and plant temperature. (Frost et al., 2008; Kim et al., 2006; Ramos-Solano et al., 2010). Stressful situations cause a variety of biochemical and physiological changes, which may affect plant metabolism, performance and yield (Xiong et al., 2002).

1.8. Reactive Oxygen Species (ROS)

The generation of ROS (Fig. 3) in plants is triggered by different kinds of environmental stressors, such as light intensity, temperature, herbicides, salinity, air pollution, drought, heavy metals, nutrient deficiency and pathogen attack (Tripathy and Oelmüller, 2012; Elstner, 1991; Malan et al., 1990). ROS are by-products of diverse metabolic pathways (photosynthesis, photorespiration and CO2 assimilation) and is localized in different cell compartments such as chloroplasts, mitochondria and peroxisomes, mostly. In response to stress signals, peroxidases and amine oxidases (present in cell walls) and NADPH oxidase (located in the plasma membrane) produce ROS (Apel and Hirt, 2004).

ROS are scavenged or detoxified by different components of enzymatic or nonenzymatic antioxidant defence system that are often confined to particular compartments, under certain physiological conditions (Alscher et al., 1997, 2002). Nonenzymatic antioxidants include the major cellular redox buffer ascorbate and glutathione (GSH). Especially mutants are hypersensitive to stress, which decreases both, ascorbic acid levels or alter GSH content (Conklin et al., 1996; Creissen et al., 1999).

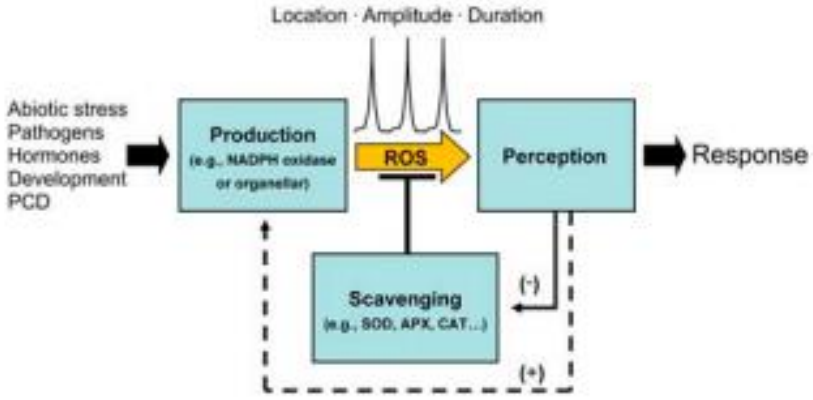


Figure 3 - ROS form in plant cells as a consequence of biotic and abiotic stress, producing a response. (Bailey-Serres, and Mittler, 2006)

A common feature among the different ROS types is their capacity to cause oxidative damage to proteins, lipids and DNA. However, ROS can also function as signaling molecules involved in defense response to pathogens and/or regulation, but mainly at very low concentrations, depending on its intracellular concentrations (Apel and Hirt, 2004).

ROS have been proposed to affect stress responses in two different ways. In one hand is the reaction of ROS in large biological molecules that causes irreversible damage, leading to tissues necrosis and ultimately causing plants death (programmed cell death) (Girotti, 2001; Rebeiz et al., 1988). On the other hand, ROS influence the expression of several genes and pathways of signal transduction related to plant defense (Apel and Hirt, 2004). These suggest that cells have developed strategies to use ROS as environmental indicators and biological signals that use and control various genetic stress response programs (Dalton et al., 1999). Whether ROS act as damaging, protective or signaling factors depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratão et al., 2005).

There are at least three major possibilities for these to act as biological signals in plants: (i) ROS may act as a second messenger and modify the activity of a specific target molecule involved in signaling or transcription; (ii) ROS may change the gene expression resulting from their cytotoxicity (polyunsaturated fatty acids within the lipids are a preferred target of ROS attack); (iii) finally, ROS may trigger stress responses in plants by modulating gene expression in a more indirect way (Foryer and Noctor, 2000; Grether-Beck et al., 2000).

There are three key enzymatic antioxidants for detoxification of ROS in chloroplasts, superoxide dismutase (EC 1.15.1.1, SOD), ascorbate peroxidase (EC 1.11.1.11, APX) and catalase (EC 1.11.1.6, CAT).

Superoxide dismutase (SOD) (Fig. 4) is the first defense line against ROS. SODs act in different compartments of the cell, such as chloroplasts, mitochondrion, microsomes, glyoxysomes and peroxisome. SOD is responsible for the conversion of hydrogen superoxide (O_2^-) in H_2O_2.

On the other hand, because hydrogen peroxide (H_2O_2) is harmful to cell, catalase (CAT), located mainly in peroxisomes, decompose hydrogen peroxide into oxygen and water, protecting the cell (Apel and Hirt, 2004).

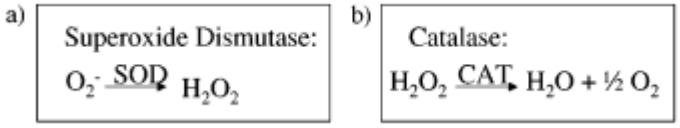


Figure 4 – The principal modes of enzymatic ROS scavenging. (a) superoxide dismutase (SOD) and (b) Catalase (CAT).

1.9. Chitosan application in crop protection

Plant pathogens induce decay on a large number of agricultural crops around the world, during de growing season and postharvest (Badawy and Rabea, 2011). Chemical pesticides supply the first mean to control plant pathogens, however, the use of such compound increase public concern regarding continuous contamination of agricultural products with pesticide residues and proliferation of resistance in pest population (Casida and Quistad, 1998; Carson, 1962; Houeto et al., 1995).

Aiming to tackle these issues, chitosan has been tested during the past decades due to its known biological properties as, immunostimulator, antibacterial, antioxidant, and antiviral polymer (Xie et al. 2001; Prashanth et al. 2007). Chitosan have been used to control disease or reduce their spread, to chelate nutrients and minerals, preventing pathogens, or to enhance plant innate defenses (El Hadrami et al., 2010).

Due to its high solubility in weak organic acids such as ascorbic acid, acetic acid, lactic acid, among others (Sajomsang et al., 2008; Silva et al., 2003; Zhao et al., 2009). Most of the characteristic properties of chitosan are due to the high content of primary amino groups with a pKa of 6.3. The positive charge, at a low pH, changes the $-NH_3^+$ groups, converting chitosan to a water-soluble cationic polyelectrolyte.

Amino groups are responsible for several straightforward chemical modifications (Kim et al., 2008; Van der Lubben et al., 2001; Alves and Mano, 2008; Clasen et al., 2006), which confers to this polymer numerous and unique physiological and biological properties with great potential in a wide range of industries such as pharmacology, medicine, and agriculture (Bautista-Baños et al., 2004; Rinaudo, 2006; Tang et al., 2010).

Some characteristics have to be present for antimicrobial polymers such as (i) easily and inexpensively synthesized, (ii) stable in long-term usage and storage at the temperature of its intended application, (iii) soluble in water or neutral media; (iv) doesn't decomposed to and/or emit toxic products, (v) should not be toxic or irritating to those who are handling it, (vi) can be regenerated upon loss of activity, and (vii) biocidal to a broad spectrum of pathogenic microorganisms in brief times of contact (Kenawy et al., 2005).

In addition, good results have been reported using chitosan as a safe alternative to pesticides with negligible risk to human health and environmental friendly (Muzzarelli, 1983). Chitosan and its derivatives have proved antimicrobial and plant-defense elicitor function (Rabea et al., 2003; Albersheim and Darvill, 1985; Benhamou and Lafontaine. 1995; El Ghaouth et al., 1992; Quintana-Obregon et al., 2011; Xia et al., 2011).

It has been demonstrated that low molecular weight chitosan's (LMWC, of less than 10 kDa) have greater antimicrobial activity than medium or high molecular weight. However, chitosan with lower molecular weight could have no activity (Uchida et al., 1989). Chitosan with a molecular weight ranging from 10,000 to 100,000 Da would be helpful containing the growth of bacteria (Seiichi et al., 1994).

Chitosan's antimicrobial activity has received considerable interest due to the problems associated with harmful synthetic antimicrobial agents (Guo et al., 2007; Roller and Covill, 1999). Their inhibition was observed on different development stages such as mycelial growth, sporulation, spore viability and germination, and the production of fungal virulence factors (Roller and Covill, 1999; Chau et al., 1996; El Ghaouth et al., 1992). Depending on the type of chitosan (native or modified), its degree of polymerization, the host, the chemical and/or nutrient composition of the substrates, and environmental conditions, chitosan exhibits a variety of antimicrobial activities (Pospieszny et al., 1991; Rabea et al., 2003; Kulikov et al., 2006).

Against viruses, chitosan proved to inhibit the systemic propagation, throughout the plant and to enhance the host's hypersensitive response to infection (Pospieszny et al., 1991; Chirkov, 2002; Faoro et al., 2001). According to the molecular weight, these have shown different level of suppression against viral infections, potato virus X, tobacco mosaic and

necrosis viruses, alfalfa mosaic virus, peanut stunt virus and cucumber mosaic virus (Pospieszny et al., 1991 and 1996; Pospieszny, 1997; Chirkov, 2002; Struszczyk, 2001).

Chitosan as proved to be a good candidate to inhibit growth of a wide range of bacteria (Muzzarelli et al., 1990). Studies carried out on *Bacillus cereus*, *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella enterica*, *B. subtilis*, *Listeria monocytogenes* and *Klebsiella pneumoniae* showed low molecular weight chitosan have great effectiveness on the reduction of growth inhibition (Jing et al., 2007; Jung et al., 2002; Omura et al., 2003; Tikhonov et al., 2006; Tsai et al, 2004; Zivanovic et al., 2004).

Against fungus, chitosan is assumed to be fungistatic with a potential to communicate regulatory changes in host and fungus (Raafat et al, 2008; Assis, 2008). Their effectiveness has been reported as inhibiting spore germination, germ tube elongation and radial growth (El Ghaouth et al., 1992; Sahai and Manocha, 1993). Similarly to the effects observed in bacteria cells, the mechanism of chitosan involves cell wall morphogenesis interacting directly with fungal growth. (El Ghaouth et al., 1992). Low chitosan concentration (20-150 mg.L-1) inhibit 80% of plan fungus such as *Phomopsis asparagi* and as high as 95% against *Fusarium oxysporum*, *Cucumernum owen*, Rhizoctonia solani and Fusarium oxysporum (Zhang et al., 2003).

1.10. Chitosan as seed soaking agent

Seed soaking is an efficient technology to enhance rapid and uniform emergence and to vigor, leading to better seedling establishment and yield. It is a simple and low cost hydration technique in which seeds are partially hydrated to a point where pre-germination metabolic activities start without actual germination, and then re-dried until close to the original dry weight (Singh et al, 2015).

Seed soaking has been shown to improvement seed performance under sub-optimal temperature conditions (Lin and Sung, 2001). Soaking increase the environmental range suitable for germination, providing faster and synchronous seedling subductions (McDonald, 1999). Previously published studies proved that seeds treated with chitosan induced resistance to certain disease(Reddy et al., 1999), and increase the energy of

germination, germination percentage, lipase activity (Zhou et al., 2002), accelerated seed germination, improved tolerance to stress condition and restrain the growth and reproduction of some bacterial and virus that are present in crops (Cheah et al., 1997).

The beneficial effects of seed priming have been demonstrated for many field crops such as wheat, sweet corn, mung bean, barley, lentil, cucumber etc. (Sadeghian and Yavari, 2004).

Table 1. Application of chitosan as seed coater, seed soaker, sprayed and in the soil used in different fruits.

Crop	Method of application	Effects	References
Peach	• Fruit coating	•Increased SOD activity	• Li and Yu (2001)
Loquat fruits	• Fruit coating	•Increased ascorbic acid and antioxidant capacity	• Ghasemnezhad et al. (2011)
Apricot	• Fruit coating	•Increased antioxidant activity	• Ghasemnezhad et al. (2010)
Artichoke	•Seed coating after harvest	• 4% (w/v) Chitosan was better for coating • Enhanced seed germination and quality • Increased plant growth	• Ziani et al. (2010)
Tomato	• Seed soaking	• Increase seedling weight • Protect from crown rot and root rot	• Li et al. (2013)
Watermelon	• Seed soaking prior to germination	• Decreased seedling death • Increased fresh and dry seedling weight	• Li et al. (2013)
Rice	• Chitosan in soil	• During seed soaking, the yield rice increased 17% in comparison to control	• Ghasemnezhad et al. (2011)
Sweet basil	• Seed soaking and root dipping before transplanting	• Increased phenolic and terpenic compounds, especially rosmarinic acid and eugenol	• Kim et al. (2005)

Researchers developed different techniques of seed soaking, which include hydropriming, halopriming, osmopriming, and hormonal priming.

Hydropriming technique it's a soaking water seed before sowing (Pill and Necker, 2001) and may or may not be followed by air drying of the seeds. This technique may enhance seed germination, radicle, plumule and seedling emergence in different crop species under both saline and non-saline conditions, as well as show a positive effect on enzyme activity required for rapid germination. Roy and Srivastava (1999) demonstrated that soaking wheat kernels in water improved their germination rate under saline conditions. It also had pronounced effect on field emergence its rate and early seedling growth of maize crop, improving the field stand and plant growth, both at vegetative and maturity of maize (Nagar et al., 1998).

Halopriming technique, soaking the seeds in solution of inorganic salts such as NaCl, KNO_3, $CaSO_4$, among others. Halo-priming promote seed germination, seedling emergence and establishment and final crop yield in salt affected soil. Khan et al., (2009) evaluated the response of seeds primed with NaCl solutions at different salinity levels 0, 3, 6 and 9 dSm-1 in relation to early growth stages and concluded that seed priming with NaCl was found to be the best treatment when compared to nonprimed seeds. Other authors proved the increase of activity in sorghum seeds soaked in KNO_3 solution of total amylases and proteases in germinating seeds under salt stress conditions (Kadiri and Hussaini, 1999).

The technique of osmopriming, soaking the seeds for a period of time in a solution of sugar, PEG, $CaCl_2$, etc followed by air drying before sowing. Osmopriming simply means soaking seeds in osmotic solution (Parera and Cantliff, 1994). Among other improvements, osmopriming enhance crop performance and seed germination, promoting high seed vigour and radical and plumule length at a low temperature, improved the seedling stand establishment parameters.

Rehman et al. (2014) studied the influence on early crop growth, phonological development and yield performance of Linola in Pakistan, treating the seeds with salicylic acid, $CaCl_2$ and Moringa Leaf Extract (MLE), including untreated seeds. Results showed that osmopriming with $CaCl_2$ reduced immersion time, produced heavier seedling fresh

and dry weight, increased chlorophyll *a* content, reduce crop branching, flowering and maturity time, resulting in the maximum plant height, number of branches, tillers, pods and seeds per pod followed by MLE. The researcher concluded that seed osmopriming with $CaCl_2$ and MLE can play a significant role in early crop growth and seed yield of linola.

Hormonal priming technique does a pre-seed treatment with different hormones like GA_3, kinetin, ascorbate etc., promoting the growth and development of the seedlings. Ashraf and Rauf (2001), found that Gibberellic acid (GA_3) treatment promote the vegetative growth of two wheat cultivars, enhancing the deposition of Na^+ and Cl^- in both root and shoots of wheat plant, increasing significantly photosynthesis at the vegetative stage of the crops.

1.11. Foliar treatment agent

Treatments with chitosan has foliar applicator has been reported in many systems and for several purposes, for example, enhance fruit weight productivity in tomato (Sathiyabama et al., 2014) and fruit yield, plant height, and leaf number (Mondal et al., 2012).

Foliar application of chitosan affect the net photosynthetic rate of soybean and maze on day after application (Khan et al., 2002), this correlated with increases in stomatal conductance and transpiration rate. One the other hand chitosan foliar application did not have any effect on the intercellular CO_2 concentration, and did not affect maize or soybean height, root length, leaf area, or total dry mass.

Bittelli *et al.* (2001) suggested that chitosan might be an effective anti-transept, preserving water resources use in agriculture. The authors discovered that stomata closure in response to treatment with chitosan, resulted in a decrease in transpiration rate at 26-43%, while there was no change in biomass production or yield (Bittelli et al., 2001). Iriti *et al.* (2009) revealed some of the aspects through which chitosan was able to reduce transpiration in bean plants after being used as foliar spray. The authors showed that this activity was likely to occur thanks to the increase in abscisic acid (ABA) content in treated leaves, occurring a partial stomatal closure, leading, among other aspects, to a decrease in conductance of water vapor and in the overall transpiration rate.

Foliar treatment with chitosan has also been extensively used to control growth, spread and development of many diseases involving viruses, bacteria, fungi and pests (Rabea et al., 2003) and increase yield and tuber quality of micropropagated greenhouse-grown potatoes (Kowalski et al., 2006).

Chapter 2: Aim of the study

The aim of this study was to evaluate the effects of chitosan on seed germination and plant growth, using two different biological models: *Solanum lycopersicum* and *Coriandrum sativum.*

In order to study biotechnological applications of this important biopolymer obtained from byproducts (crustacean shells) , it were performed some experiments namely:

(1) Chitin extraction from *Penaeus monodon* shell, evaluation the mineral content in terms of ash and chitin production and evaluation of chitosan content;

(2) Production of low molecular weight chitosan and physicochemical characterization (molecular weight and polydispersity index);

(3) Chitosan effects evaluation on *Solanum lycopersicum* and *Coriandrum sativum* , in terms of rate germination, water loss by leaves and plant growth;

(4) Assessment of the oxidative degradation of the lipids caused by an oxidative stress in order to understand if chitosan affects the membrane permeability.

Chapter 3: Materials and methods

3.1. Sample collection and processing

The shrimp samples of "*Penaeus monodon*" were provided by the company Altakitin, S.A. The organisms were dried at room temperature and the raw material was powdered and sieved into particles between 150 and 500 μm (diameter).

3.2. Chitin extraction and chitosan production

3.2.1. Chitin extraction

In order to remove the chitin content present in the exoskeleton, two major constituents of the shell were removed first: the proteins, removed through deproteinization and the inorganic calcium carbonate, removed through demineralization. Their order may be reversed with some benefit, especially when enzymatic treatment is considered (Dutta et al., 2004; Ospina Álvarez et al., 2014).

Raw samples were treated with a 0.5M chloridric acid (HCl) solution, with a 1:30 (w/v) ratio, at 21˚C, during 10 min. After demineralization, the raw material was subjected to several washes with distilled water to dilute the remaining acid, until neutral pH and then dried at 60˚C, with aeration, during 2 days.

The drying process was followed by a deproteinization step. The treatment was carried out with a 1M NaOH solution at a ratio of 1:15 (v/w) for 4 hours at 80˚C in a water-bath. The solution was stirred out every 30 minutes. Subsequently, the samples were washed with distilled water until the wash became colorless with a neutral pH.

3.2.2. Chitosan production

The last step to produce chitosan was deacetylation. The chitin obtained was subjected to a 12M NaOH solution at a ratio of 1:15 (v/w) at 120˚C during 8 hours. The solution was maintained on a water-bath and was stirred every 30 minutes.

After cooling at room temperature, the chitosan was filtered, washed with distilled water and dried in an oven for 48 hours at 50˚C with permanent aeration.

3.2.3. Chitosan purification

To guarantee the complete dissolution of chitosan, the samples had to be purified by a filtration method. The purification process was designed in two steps: removal of insoluble contents through filtration and precipitation of chitosan with 1M sodium hydroxide (NaOH) (Qian and Glanville, 2005).

To dissolve the soluble content, 50 ml of acetic acid 1% (v/v) were used per gram of chitosan. The insoluble content was removed by filtration with a Whatman filter paper (Whatman™ 1001-110 Grade 1 Qualitative Filter Paper, Diameter: 11cm, Pore Size: 11µm).

The chitosan solution was then precipitated by titration with 1M NaOH until no more reaction was observed. After being washed several times with distilled water, the chitosan products were centrifuged at 10,000 rpm for 5 minutes.

3.2.4. Mineral content

The ash content was determined after deacetylation to evaluate the efficacy of the process. In this process, weighted samples were subjected to combustion. The samples were then placed in a furnace at 530˚C during 20 hours. The remaining material was weighted after cooling in a desiccator.

$$\text{Ash\%} = \frac{W2 - W0}{W1 - W0} \times 100$$ (Bumgardner et al., 2003)

where W_0 is the constant weight of crucible, W_1 is the weight of sample and crucible, W_2 is the weight of ash and crucible.

3.3. Chitosan chemical characterization

3.3.1. Low molecular weight chitosan

To obtain low molecular weight products, chitosan samples (2g) were placed in a goblet and dissolved in 100 mL of acetic acid 1%. After complete dissolution, hydrogen peroxide (H_2O_2) at 5% was added.

The reaction mixture was placed in a microwave for 4 minutes at a power level of 1040 W. The solution was left to cool at room temperature and later, placed in an oven with aeration at 50°C until a dry film was formed.

The film (2g) was suspended in 100 mL of acetic acid 1% (v/v) and mixed for half an hour. After being completely dissolved, 1M NaOH was used to precipitate the low molecular weight products. Through centrifugation (10000g, 5 min), the pellet was collected. The pH was adjusted to 7 with milli-Q water.

Finally, the low molecular weight chitosan was lyophilized, forming a powder.

3.3.2. Molecular weight

The molecular weight was determined by Gel permeation chromatography (GPC). Pullulans with a molecular weight range from 1.25×10^5 to 10.05×10^5 (American Polymer Standards Corporation, Mentor, OH, USA) were used as standard to establish the calibration curve.

The GPC equipment used was a KNAUER (Advanced scientific instrument) and consists of a GPC/SEC column advancing polymer solution (column temperature is approximately 30°C) and the software used was Clarity Chromatography station for windows (V. 2.6.2.226).

All chitosan solutions with final concentration of 2 mg/mL were prepared with 0.18M acetic acid/ $3{,}85 \times 10^{-4}$ M sodium azide, being 100µL of each sample injected into GPC, with a flow rate of 1 mL/min with a range of 6.000Mw – 805.000Mw and continuous phase of 0.2M acetic acid/ 0.1M sodium acetate at an approximate pH of 5. All the solvents and solutions were filtered through a 0.45 µm filter (Watman Inc., NJ, USA).

3.4. Chitosan seed soaking experiments.

For testing the efficacy of chitosan for seed soaking, *Solanum lycopersicum* and *Coradrium stavium* seeds were used. Seed soaking was divided in 2 experiments, in order to obtain the optimal time to ensure major development of the seeds.

The solution used in this experiment was ascorbic acid at 2,5% with and without chitosan and acetic acid at 1% with and without chitosan at low molecular weight. The solutions with chitosan were prepared at a concentration of $10g.L^{-1}$.

For the first experiment, twenty-eight seeds of each species were immersed in different solutions and for the second experiment only fourteen seeds were used. First, the seeds were immersed in the solutions for half an hour and for the second experiment, the seeds were immersed for 3 hours (Suvannasara and Boonlertnirum, 2013).

On the first experiment, the control seeds of *S. lycopersicum* and *C. sativum.,* were planted directly on soil, without exposure to water. The second experiment, the control seeds of *S. lycopersicum.* and *C. sativum,* were first soaked indistilled water for 3h under stirring, matching the same time as the other seeds in different conditions.

3.5. Experimental conditions

3.5.1. Soil

The soil samples used (SIRO) were previously autoclaved to remove all the microorganisms that could increase the variables in study. After the soil was sterilized, vermiculite was added at a ratio of approximately 70:30 so that more oxygen was available and granting an easier capture of soil nutrients by the plant roots (Blankendaal, 1972).

The soils were checked every day in terms of pH and relative humidity, ensuring that these ranged from 6,5 to 7 and 40 to 50% for pH and relative humidity, respectively (Shaxson and Barber, 2016).

3.5.2. Irrigation system

At the start of the experiment, the seeds were watered the first 3 days with 250 ml, on the tray, and 10 ml on the pot so that the soil couldproperly retain the water. After that, the seeds were watered every three days, always with 250 ml of water on the tray.

3.5.3. Climatic chamber and conditions

The climatic chamber was set to 25°C, with a luminosity of approximately 26300 lux, with a relative humidity of 50% and a photoperiod of 14h of light and 10 hours of dark (Blankendaal, 1972).

The first experiment took place over the course of 30 days and the second experiment over the course of 3 weeks. During the last day of the experiment, the temperature was changed to 40°C for the daylight hours.

3.6. Chitosan plant effects

3.6.1. Germination rate

The germination rate was calculated in order to obtain a greater perception of the number of plants that could be obtained with and without chitosan. In this way, the germination rate was calculated using the following formula:

$$\%\text{Germination rate} = \frac{\text{number of seeds that germinated}}{\text{number of seeds planted}} \times 100$$

3.6.2. Water loss by leaves

Transpiration is the process of moving water through the aerated parts of the plant such as leaves, stems and flowers. The leaves have on their surface pores called stomata that open and close, facilitating gas exchange. Stomach opening has an associated "cost" since it loses water every time these pores are opened.

In this experiment the loss of water in the leaves at different times wasperformed, being recorded the initial weight after 30 minutes, 2 hours, 4 hours, 6 hours and after 84 hours,

in order to verify if the chitosan has influence over leaf transpiration caused by high temperatures. In the first experiment the water loss was made on the 15th and the 30th day after planting, being the number of replicates 12 and 6 respectively, and for the 2nd experiment the loss of water on the leaves was done on the 21st day, being the number of replicas 4.

The oven temperature was set to 33 ° C, so that the transpiration was constant.

3.6.3. Development and growth of the roots and stem

Embryo germinates from its seed and begins to produce additional organs (leaves, stems, and roots) through the process of organogenesis. New roots grow from root meristems located at the tip of the root, and new stems and leaves grow from shoot meristems located at the tip of the shoot (Brand et al., 2001).

The development and growth of the roots and stem of the plants was registered after 15 and 30 days in the first experiment, with 8 replicates each and at the end of 21 days for the 2nd experiment with 4 replicates. The measurements of the plants were made with a normal ruler at the times indicated above, and the length of roots and stem of the plants was recorded.

3.7. Lipid peroxidation (LPO)

The level of lipid peroxidation in both species was measured in terms of Malondialdehyde (MDA) concentration, according to the method of Velez et al., 2016. Fresh leaves were homogenized with trichloroacetic acid (TCA) at 20% (1ml). For all the conditions tested, 4 replicates were processed, being weighed 0,2g of leaves per sample.

Then, homogenized leaves were centrifuged at 10.000g during 10 minutes at 4°C. The supernatant was then recovered, while the pellet was discarded. The supernatant was stored at -80°C for 24h.

Afterde frosting in a refrigerator (4ºC) , 400 µl were removed from the supernatant of each sample and added to 400 µl of thiobarbituric acid (TBA) at 0,5%. The blanc was prepared

with 400 µl of TBA plus 400 µl of TCA. The samples (included the blanc) went to the oven at 96°C during 25 minutes.

Afterwards, the samples were placed into ice to stop the reaction. From each sample, 200µl were transferred into 96-well microplate, being all conditions performed in triplicate.

$A = Ɛ \times b \times C$;

where Molar extinction coefficient (Ɛ) is $1.56 \times 10^5 M^{-1}.cm^{-1}$, Absorbance (A) is 535 nm, b is 1 cm and C is the concentration in Molar.

3.8. Statistical analysis

The data for water loss, root and stem growth and lipid peroxidation obtained were subjected to analysis of variance (ANOVA). Significance was assessed at the 95% level throughout. For the water loss by leaves, root and stem growth and lipid peroxidation the statistical method used was Dunnett's method, being analyzed with a general linear model unvaried, comparing all conditions with control.

IBM SPSS Statistics 23 statistical software was used for analysis and graphics.

Chapter 4: Results and discussion

4.1. Raw material characterization

The main sources of raw material for the production of chitin are the exoskeletons of various crustacean species, mainly crabs and shrimps (Younes and Rinaudo, 2015). The shellfish of crustaceans are made between a complex and very intimate network with proteins and chitin onto which calcium carbonate deposits to form the rigid shell (Horst et al., 1993).

The biochemical composition of raw material (percentage of ash and chitin) of the shrimp exoskeleton, *Penaeus monodon*, is showed in table 2.

Table 2. Characterization of dried samples of *Penaeus monodon* (exoskeleton) expressed as the mass percentage of the initial dried material. Values are means of 5 replicates for ash percentage and three replicates for chitin percentage. The results are mean ± standard deviation.

Raw Material	Ash (%)	Chitin (%)
Exoskeleton	0.7 ± 0.17	13.6 ± 1.22

According to the results obtained (Table 2) shrimp shell contains a percentage of ash value of 0.7 ± 0.17%, being correlated directly with the mineral content in the sample. According to Wang and Kinsella (1976), the commercial chitosan sample have an ash value of about 1.18%. This result demonstrate that the percentage of mineral content in samples are in accordance to the results of the supposed chitosan commercialized. The chitin production was 13.6 ± 1.22.

After demineralization, deproteinization and deacetylation steps, the yield of chitosan in percentage it was 10.85 ± 0.21%. According to Yen et al, (2009), the results showed a very low value, being the percentage yield of chitosan in the range of 30.0-32.2%.

4.2. Chitosan and polymer physicocharacterization

To estimate the molecular weight distribution, its weight-average molecular weight (Mw) and number-average molecular weight (Mn) were measured by GPC. Therefore in table 3, the molecular weights and is polydispersity are summarized.

According to Ribeiro et al. (2014) low-molecular weight chitosan have an average molecular weight of approximately 120kDa, while medium Mw have an average of 250 kDa and high Mw 340 kDa, respectively. The data presented in Table 3 proved a Mw of 56.5 kDa, regarding low-molecular weight samples, being considered within range, as previously mentioned.

Table 3. Quitosan molecular weight by GPC and ratio of Mw/Mn of chitosan.

GPC		
Mw	Mn	PD
56546	17217	3.284

Polydispersion index (PD) indicates how the particle size distribution is spread. This parameter is obtained from the ratio of average weight and average molecular weight (Mw/Mn) and a value between 0.85 and 1.15 is considered as having good polymer homogeneity (Szymańska and Winnicka, 2015). Small PD values are related to narrow size distributions (Lemarchand et al., 2003), being roughly related to the purity of the polymer. According to the polydispersity obtained of 3.284, indicates that chitosan purity was not homogeneity, having different polymer size distributions.

4.3. Experiment1

4.3.1. Plant Germination

Seed germination is controlled by a number of mechanisms and is necessary for the growth and development of the embryo, resulting in the eventual production of a new plant (Miransari and Smith, 2014).

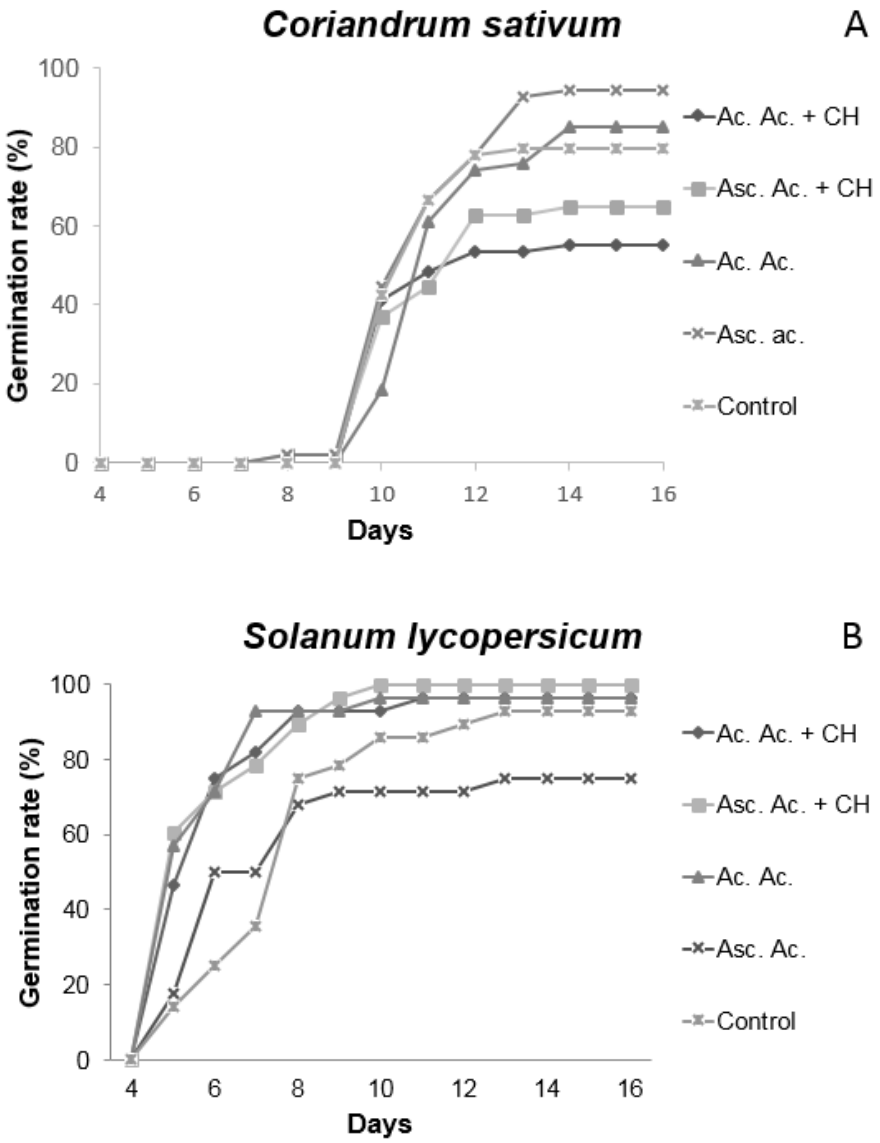


Figure 5 - *C. sativum* (A) and *S. lycopersicum* (B) response to ascorbic acid, acetic acid and chitosan priming treatments. Germination rate during 30 days growth.

The germination rate (%) (Fig. 5) showed slightly differences between conditions. For *C. sativum* (Fig. 5A), the condition ascorbic acid showed more percentage of plants germination (94.4%) after 14 days, being the most successfully condition. The condition acetic acid have a germination rate of 85.2% after 14 days. The condition control had a germination rate of 79.6% after 13 days. The two last condition, ascorbic acid and acetic acid, with chitosan had the less percentage of germination rate, being 64.8% and 55.4%, respectively.

For *S. lycopersicum* (Fig. 5B), the condition ascorbic acid with chitosan showed a 100% germination rate after 10 days of germination, having all the seeds planted, grown and germinated. The condition acetic acid and acetic acid with chitosan had a final germination rate of 96.4% after 10 days. The control condition reaches the maximum number of seeds germinated at 13 days (92.9%). The condition ascorbic acid had a percentage of 75% on the 13 day of germination, being the condition that grown less plants comparing to the other conditions already mentioned.

According to the results obtained the plants of *C. sativum* looks like to be an negative response to chitosan, once that showed less germination percentage of plants when chitosan are present. On the other hand, *S. lycopersicum* showed an positive response to chitosan taking more plants germinated when chitosan are present.

4.3.2. Water loss of plant leaves

Water stress (WS) in plants causing by drought is a major problem in agriculture productivity, especially in tropical, semi-arid and arid areas of the world. Mediterranean regions are the best climate model, indicating substantial drying and warming in some cases (Giorgi and Lionello, 2008).

In the present study, water loss by detached leaves of *C. sativum* and *S. lycopersicum* after 15 days at the start of the experiment is showed.

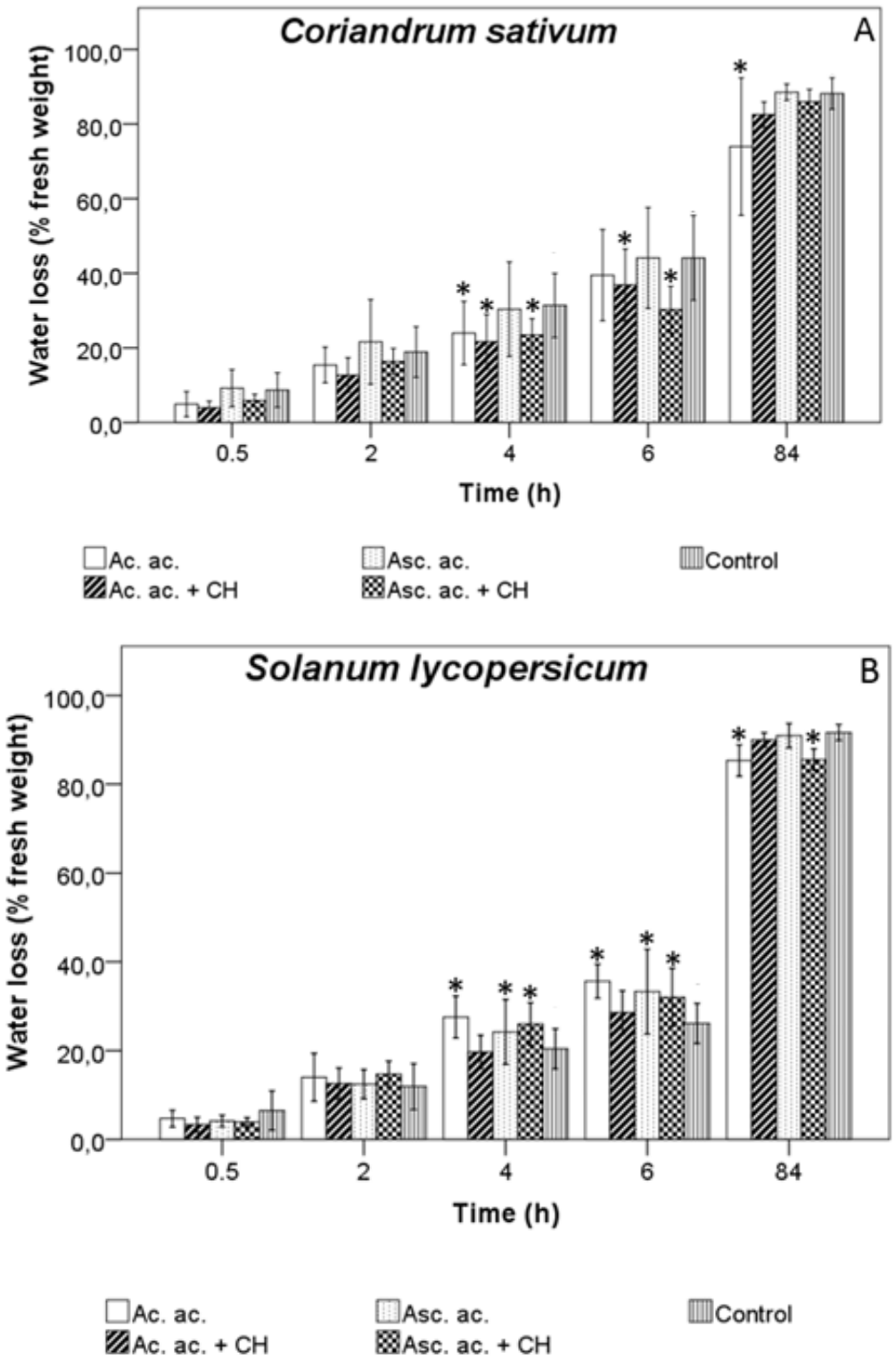


Figure 6 - Water loss by leaves of *C. sativum* (A) and *S. lycopersicum* (B) under stress condition caused by the fact of being detached from the stem and under hot conditions. Data are means ± Standard deviation (n=12). Asterisks indicate significantly different between the control and the conditions determined by Dunnett's *t* test ($0.05 \leq P < 0.01$).

The leaves of *C. sativum* and *S. lycopersicum* were submitted to equal temperatures, in order to obtain which condition offers greater capacity of retention of water in it's tissues during 84h of stress.

The research showed a linear increase as expected. For *C. sativum* (Fig. 6A) in the first 30 min the conditions lost more or less the same amount of water, between 4% and 9% to acetic acid with chitosan and ascorbic acid, respectively. At 2 hours the condition acetic acid with chitosan lost less water by the leaf (12.7%) and the condition ascorbic acid is the condition that lost more water (21.6%). After 4 hours at the beginning of the experiment, the differences among them are more notable, being once that the conditions with chitosan lost less water than the conditions without chitosan and control. After 6 hours, the ascorbic acid with chitosan lost 30.3% of water content, being the condition, at this time that lose less water. For the other hand, the conditions ascorbic acid without chitosan and control lose more water (44.1%) than the other conditions. At the end of the experiment, the condition acetic acid lost less water (74%) than the other conditions, retaining water in their leaf tissues.

For *S. lycopersicum* (Fig. 6B) the water content present in leaves at the first 2 hours are more or less the same, being that all conditions retain in their tissues the content of water necessarily for their metabolic processes. After 4 hours, starts to had differences between conditions, being the condition acetic acid with chitosan the condition that lost less water (19.7%) and the same condition without chitosan that lost more water content (27.6%). At 6 hours the conditions control and acetic acid with chitosan (26.1% and 28.6%, respectively) are the conditions that lost less water, for the other hand the conditions without chitosan lost more water. For acetic acid without chitosan the total water content lost was 35.6% and for ascorbic acid without chitosan the total water content lost was 33.3%. At the end of the experiment, the leaves were weighted and the total amount of water lost by the conditions ascorbic acid with chitosan and acetic acid without chitosan was 85.5% and 85.3%, respectively. The condition control lost the major content of water at this point (91.7%) than the other conditions.

Comparing the two species (Fig. 6A and 6B), the resuts confirm that the leaves that were subject to chitosan had more retention of water in their tissues during the first 6h. This results reveal to that the plants that were subject to chitosan would hold on more time

without water and can stand hot temperatures. After 84 hours the best results for less water loss is the condition acetic acid for both species.

After 15 days, the same procedure was made submitting all the seeds a certain temperature and see if retention of water is the same than 15 days earlier.

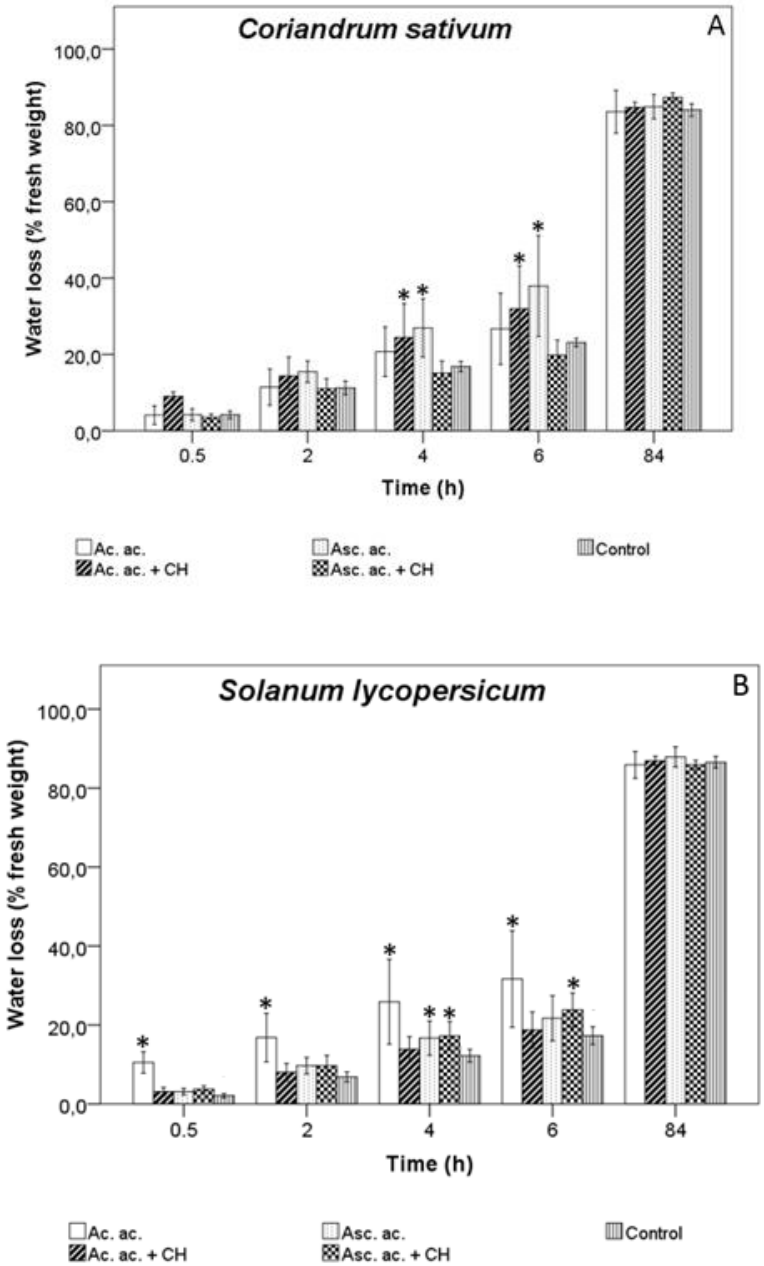


Figure 7 - Water loss by leaves of *C. sativum* (A) and *S. lycopersicum* (B) under stress condition caused by the fact of being detached from the stem and under hot conditions. Data are means ± Standard deviation (n=6). Asterisks indicate significantly different between the control and the conditions determined by Dunnett's *t* test (* $0.05 \leq P$).

On the first 30 min the amount of water lost by the leaves of *C. sativum* (Fig. 7A), are more or less equal each other, being that the condition ascorbic acid with chitosan lose 3.5% of water content and the condition acetic acid with chitosan lost 9.0%, being that last condition, that lose more water in the first 30 min. After 2 hours at the start of the experiment, the conditions acetic acid with chitosan and ascorbic acid without chitosan are the two conditions that lost more content of water (14.3% and 15.5%, respectively). The other three conditions lost more or less the same amount of water at this point. After 6 hours as usual and normal, starts to had an unbalance between conditions, being the condition ascorbic acid with chitosan, the condition that lost less water (19.8%) and the same condition without chitosan, the condition that lost more content of water (37.9%). 84 hours later at the start of the experiment, the water content lost by the leaves are more or less the same between conditions.

The results for S. lycopersicum, showed in figure 7B, reveal for the first 2 hours a near percentage of water loss between them except the condition acetic acid that lost 10.5% on the first 30 min and 16.8% after 2 hours, being this condition that reveal a water content lost bigger at this time. After 4 hours, the conditions control and acetic acid with chitosan are the conditions that lost less water (12.2% and 13.9%, respectively) and once the condition acetic acid without chitosan that lost more water (25.9%). 6 hours after the start of experiment, the same conditions (control and acetic acid with chitosan) remain the conditions that lost less water and the condition acetic acid without chitosan that lost more water. In the end of the experiment, the conditions balance between them, losing more or less the same amount 84 hours after the start of the experiment.

On figure 7A the results showed that the best two conditions after 6h was ascorbic acid with chitosan and the control, and for the figure 7B was acetic acid with chitosan and control. This results reveal that the leaves of *C. sativum* had more water retention at the conditions ascorbic acid with chitosan and for the leaves of *S. lycopersicum* was acetic acid with chitosan.

4.3.3. Plant growth

Chitosan has been reported to stimulate the immune system involved in plant resistance to pathogen infection (Pichyangkura and Chadchawan, 2015). In addition, previous

studies suggest the use of chitosan as a stimulator for vegetative growth, plant defense, crop yield and quality of vegetable crops, such as in orchid (Nge et al., 2006), faba bean (El-sawy et al., 2010), cucumber (Sheheta et al., 2012) and corn (Suvannasara et al., 2011; Lizárraga-Paulín et al., 2011), when used in the right amount. In the present study, root and shoot length of *C. sativum* and *S. lycopersicum* after 15 days is displayed in table 4 and 5.

The constituents of *C. sativum* (Root, stem and the total plant) were measured and all conditions were compared with control (table 4). Different solutions promote the root and stem growth (cm) of *C. sativum* at different rates. Comparing the root length, it is notable that Ascorbic acid and Acetic acid with chitosan (4.1 ± 0.90 cm and 4.7 ± 1.17 cm, respectively) had a significant positive influence on root growth, when compared to the same solutions without chitosan.

For stem length the chitosan doesn't showed any effect of *Coriandrum* growth. In regards to stem length, treatment with ascorbic acid (6.6 ± 0.51 cm) has showed to be more effective, promoting stem development in *C. sativum*.

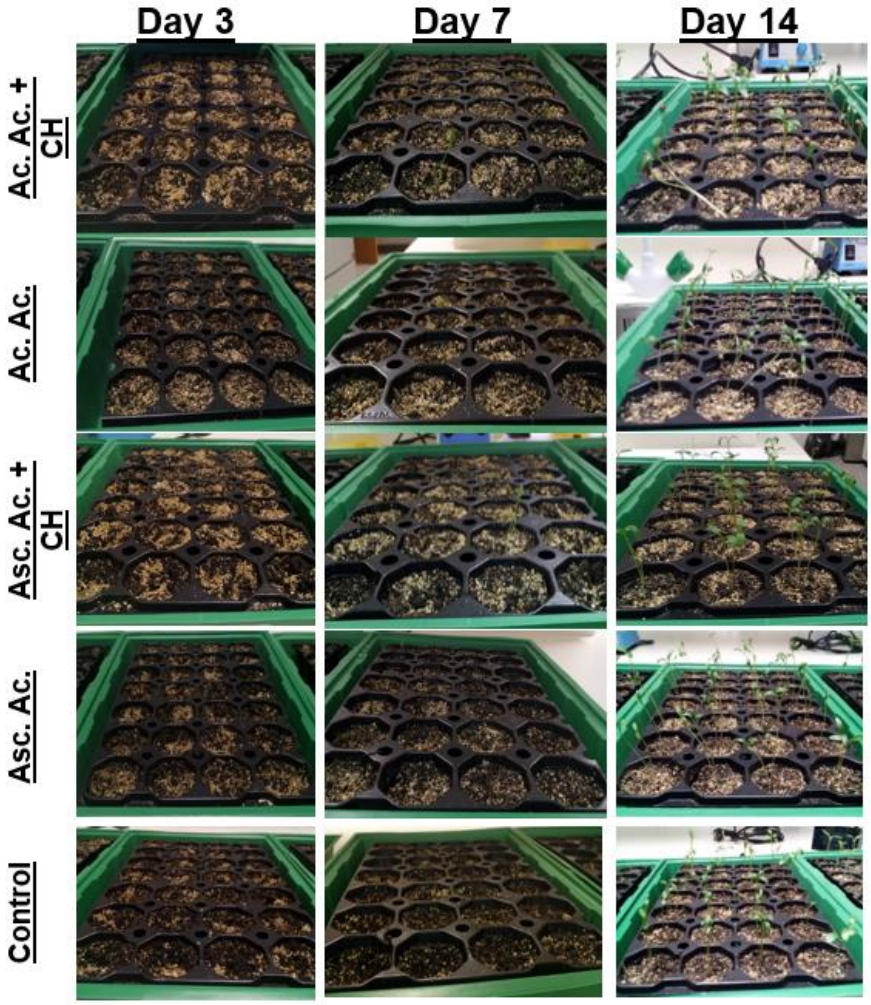


Figure 8 - Seedlings of *Coriandrum sativum* after 3, 7 and 15 days of culture.

On total plant acetic acid with chitosan (10.1 ± 1.64 cm) had more root and stem growth, comparing to acetic acid (7.3 ± 1.21 cm) condition, being this condition the worst condition registered for all the constituents of *C. sativum* after 15 days of experiment.

Table 4. Root and stem length (cm) of *C. sativum* after 15 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=8). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Coriandrum sativum			
	15 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	4.0 ± 1.23 $_{a}$	4.5 ± 1.09 $_{a}$	8.6 ± 1.58 $_{a}$
Asc. Ac. + CH	4.1 ± 0.90 $_{b}$	5.1 ± 0.68 $_{b}$	9.2 ± 1.15 $_{b}$
Asc. Ac.	3.3 ± 1.17 $_{b}$	6.6 ± 0.51 $_{a}$	9.9 ± 1.60 $_{b}$
Ac. Ac. + CH	4.7 ± 1.17 $_{b}$	5.4 ± 0.62 $_{b}$	10.1 ± 1.64 $_{b}$
Ac. Ac.	2.5 ± 0.81 $_{a}$	4.8 ± 0.93 $_{b}$	7.3 ± 1.21 $_{b}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: $0.05 \leq p < 0.01$.

On table 5, the roots of *S. lycopersicum* react when acetic acid with and without chitosan are available in the medium (7.2 ± 0.58 cm and 7.4 ± 1.19 cm, respectively), being this two conditions more efficient for plant development.

For stem growth, the conditions acetic acid and acetic acid with chitosan, reveal to inhibit the stem of *S. lycopersicum*, showing a length of 3.1 ± 0.37 cm for acetic acid and 2.6 ± 0.15 cm for acetic acid with chitosan.

Comparing the control plants to the conditions with chitosan on the total plant growth, it's notable that chitosan had influence on plant, promoting development.

Table 5. Root and stem length (cm) of *S. lycopersicum* after 15 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=8). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Solanum lycopersicum

	15 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	3.3 ± 0.85 $_{a}$	4.4 ± 0.54 $_{a}$	7.7 ± 0.99 $_{a}$
Asc. Ac. + CH	5.4 ± 1.05 $_{a}$	3.7 ± 0.33 $_{a}$	9.1 ± 1.12 $_{a}$
Asc. Ac.	3.6 ± 0.44 $_{b}$	4.0 ± 0.53 $_{b}$	7.3 ± 0.64 $_{b}$
Ac. ac. + CH	7.2 ± 0.58 $_{a}$	2.6 ± 0.15 $_{a}$	9.8 ± 0.71 $_{a}$
Ac. Ac.	7.4 ± 1.19 $_{a}$	3.1 ± 0.37 $_{a}$	10.6 ± 1.07 $_{a}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: $0.05 \leq p < 0.01$.

After 15 days the differences between *C. sativum* (table 4) and *S. lycopersicum* (table 5) exist in some treatments, being the condition Acetic Acid with chitosan the condition that visualized more growth for *C. sativum* (10.1 ± 1.64 cm) and the condition Acetic acid the condition that promoted more development to *S. lycopersicum* (10.6 ± 1.07 cm). The results after 30 days are displayed in table 6 and 7.

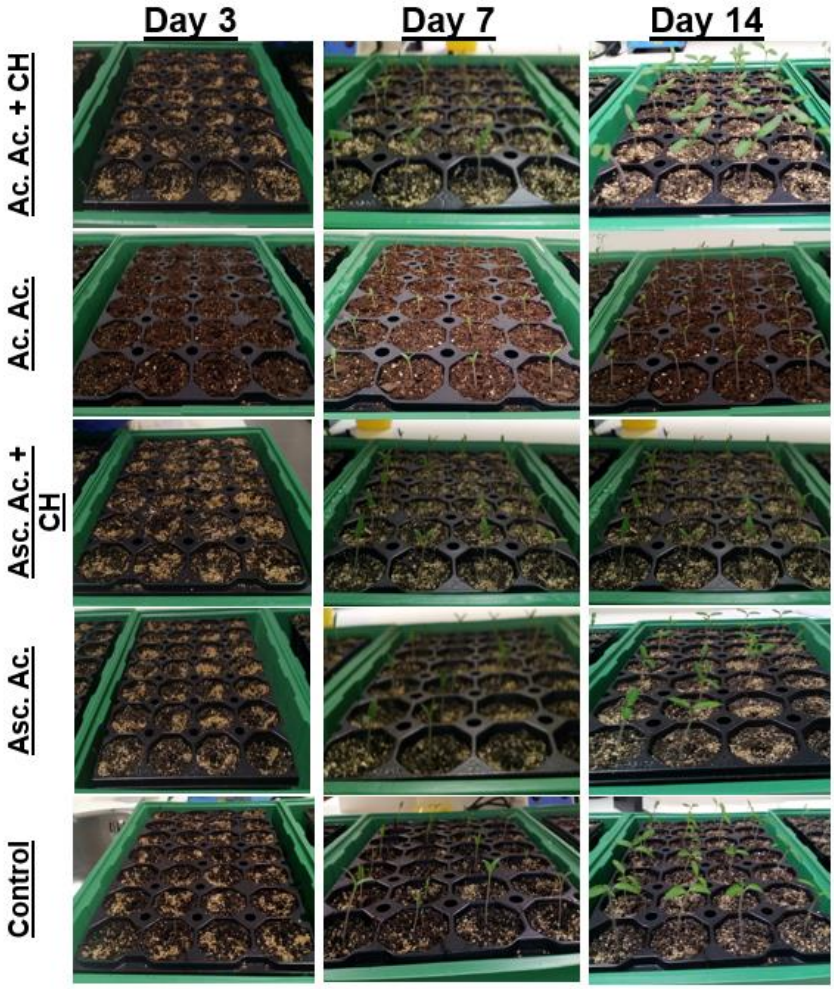


Figure 9 - Seedings of *Solanum lycopersicum* after 3, 7 and 15 days of culture.

After 30 days, the *C. sativum* root length (Table 6) of all treatments had more or less the same growth, being the conditions acetic acid without and with chitosan (6.1 ± 1.35 cm and 6.1 ± 1.00 cm) more efficient for root development.

For stem length, the conditions with chitosan doesn't showed influence on the plant development, being ascorbic acid treatment that proved to be more promotive for plant growth after 30 days (6.1 ± 0.92 cm).

Table 6. Root and stem length (cm) of *C. sativum* after 30 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=8). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Coriandrum sativum

	30 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	5.3 ± 1.27 $_{a}$	5.1 ± 0.85 $_{a}$	10.4 ± 1.60 $_{a}$
Asc. Ac. + CH	5.8 ± 1.03 $_{b}$	4.6 ± 0.88 $_{b}$	10.4 ± 1.29 $_{b}$
Asc. Ac.	6.0 ± 0.65 $_{b}$	6.1 ± 0.92 $_{b}$	12.1 ± 1.38 $_{b}$
Ac. Ac. + CH	6.1 ± 1.00 $_{b}$	5.4 ± 0.59 $_{b}$	11.5 ± 1.30 $_{b}$
Ac. Ac.	6.1 ± 1.35 $_{b}$	5.9 ± 1.03 $_{b}$	11.9 ± 2.22 $_{b}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: $0.05 \leq p < 0.01$.

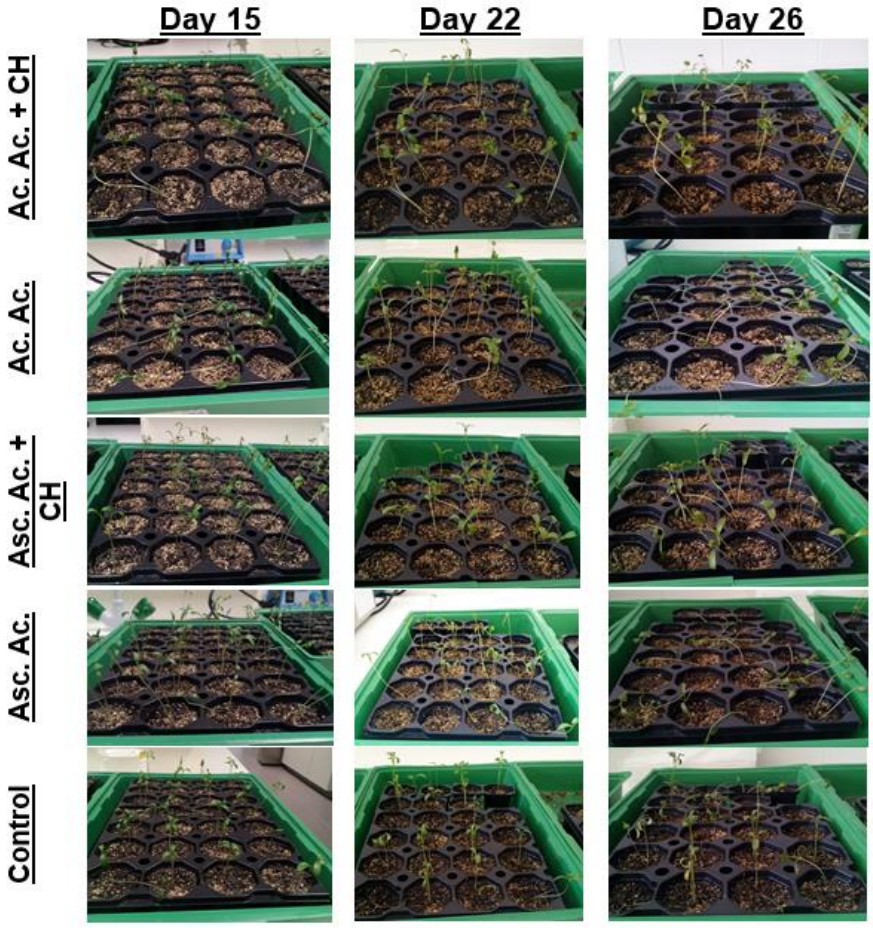


Figure 10 - Seedilngs of *Coriandrum sativum* after 15, 22 and 26 days of culture.

The results showed for *S. lycopersicum* (Table 7), reveal that roots might not be affected to the chitosan, highlighting the condition acetic acid for the remaining treatments, being this condition more effective for root growth (9.5 ± 1.77 cm).

For stem length, the chitosan conditions showed more capacity to growth the stem of plant than the conditions that doesn't had chitosan, but the most productive in terms of growth was suprisingly the control (4.6 ± 0.45 cm).

Table 7. Root and stem length (cm) of *S. lycopersicum* after 30 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=8). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Solanum lycopersicum			
	30 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	6.3 ± 1.61 $_{a}$	4.6 ± 0.45 $_{a}$	10.9 ± 1.57 $_{a}$
Asc. Ac. + CH	7.2 ± 1.84 $_{b}$	4.4 ± 0.67 $_{b}$	11.6 ± 1.90 $_{b}$
Asc. Ac.	6.1 ± 2.32 $_{b}$	3.8 ± 0.38 $_{a}$	9.9 ± 2.45 $_{b}$
Ac. ac. + CH	6.0 ± 1.64 $_{b}$	4.5 ± 0.46 $_{b}$	10.5 ± 1.82 $_{b}$
Ac. Ac.	9.5 ± 1.77 $_{a}$	3.0 ± 0.32 $_{a}$	12.5 ± 1.67 $_{b}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: $0.05 \leq p < 0.01$.

At the end of the experiment the plants reach the maximum length of 12.1 ± 1.38 cm for *C. sativum* in Ascorbic acid treatment and for the plants of *S. lycopersicum* the condition Acetic acid showed to be more efficient in terms of growth, promoting a maximum length of 12.5 ± 1.67 cm after 30 days of experiment.

Comparing the results for the total growth of the *C. sativum* specie after 15 days with the 30 days of experience, it's possible to see that for the ascorbic acid condition with chitosan the increment was only 1.2 cm and for the acetic acid condition with chitosan the increment was 1.4 cm. The highest increments were recorded for the conditions ascorbic acid without chitosan and acetic acid without chitosan, obtaining 2.2 cm and 4.6 cm, respectively.

In the *S. lycopersicum* specie, the increment for ascorbic acid with chitosan was 2.5 cm and for acetic acid with chitosan the increment was only 0.7 cm, being the latter result showing lower growth. The result obtained for the ascorbic acid without chitosan was 2.6 cm and for acetic acid without chitosan was 1.9 cm, having the conditions without chitosan

a greater growth in 15 days than the conditions with chitosan. The control was the condition that presented a greater increase for the *S. lycopersicum* specie of 3.2 cm.

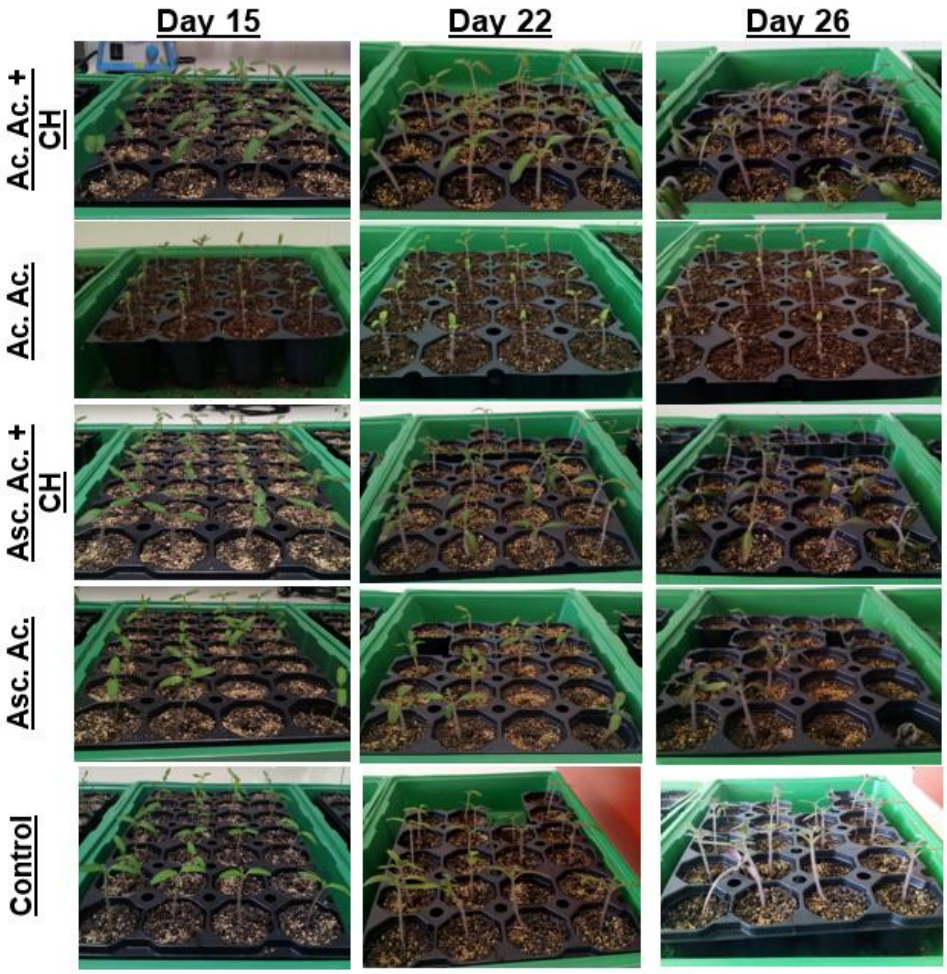


Figure 11 - Seedlings of *Solanum lycopersicum* after 15, 22 and 26 days of culture.

4.4. Experiment 2

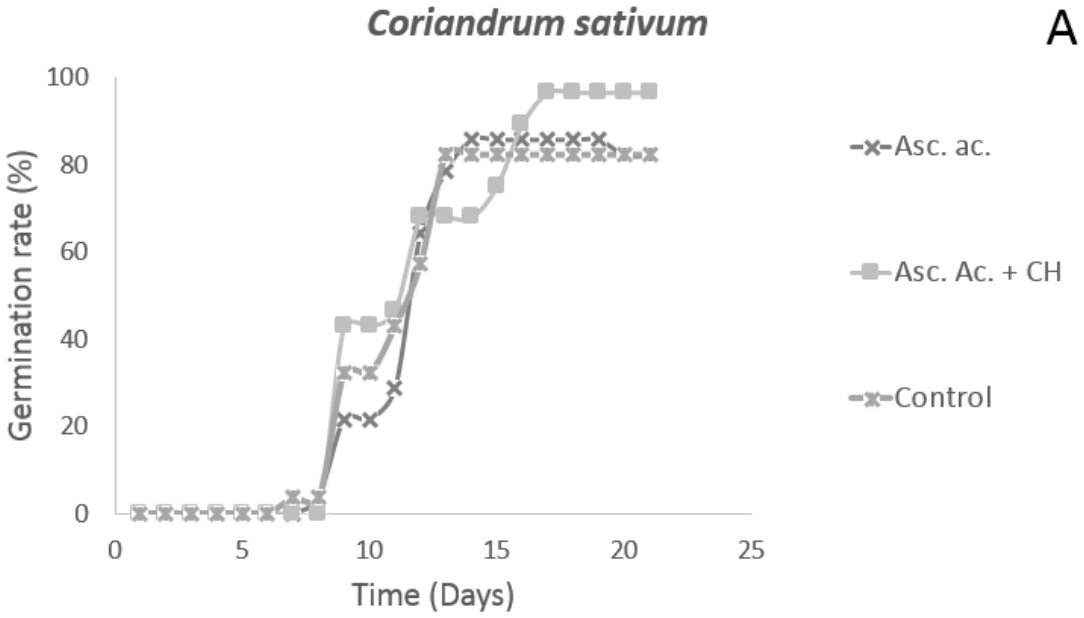
4.4.1. Plant Germination

Seed soaking for 12–24 h is known to be an efficient means for improving the germination of *Solanum* species (Hayati et al., 2005 ; Ahmed et al., 2006), as it may remove seed germination inhibitors (Bewley et al., 2013).

The results showed that the plants of *C. sativum* (Fg. 12A) doesn't develop when the seeds are dipped in acetic acid with and without chitosan during 3 hours. The germination process occurs more efficiently in control, developing the first plant after 6 days. After 21 days of experiment, ascorbic acid with chitosan stood out, having great results compared

to the other conditions (96-97%).The second conditions that achieve the best results was ascorbic acid without chitosan (85-86%) and the last condition was control (82-83%)

Various methods of presowing seed treatment have been reported to improve plant stand establishment and yields (Andreoli and Khan, 1997). In the case of *Coriander* crop the increase in germination capability and also in leaf yield as noted after seed permeation with gibberellic acid (Badgujar and Warhal, 1988; Banafar, 1994). Previous investigation by Dąbrowska et al. (1999) evidenced an increase both in seedling vigour and in yield due to seed soaking.



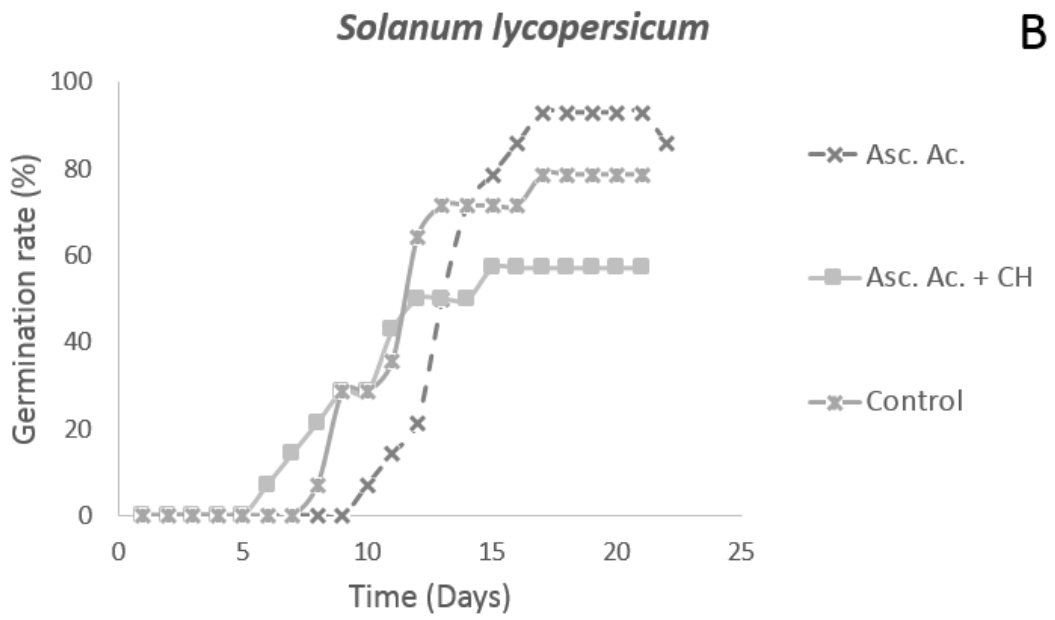


Figure 12 - *C. sativum* (A) and *S. lycopersicum* (B) response to ascorbic acid, acetic acid and chitosan priming treatments. Germination rate during 21 days growth.

The plants of *S. lycopersicum* (Fig. 12B) starts to germinate after 5 days. The seeds that were submitted to the ascorbic acid with chitosan condition were the first to germinate, the control seeds germinate in the next day and the seeds submitted to the condition ascorbic acid were the last seeds that germinate.

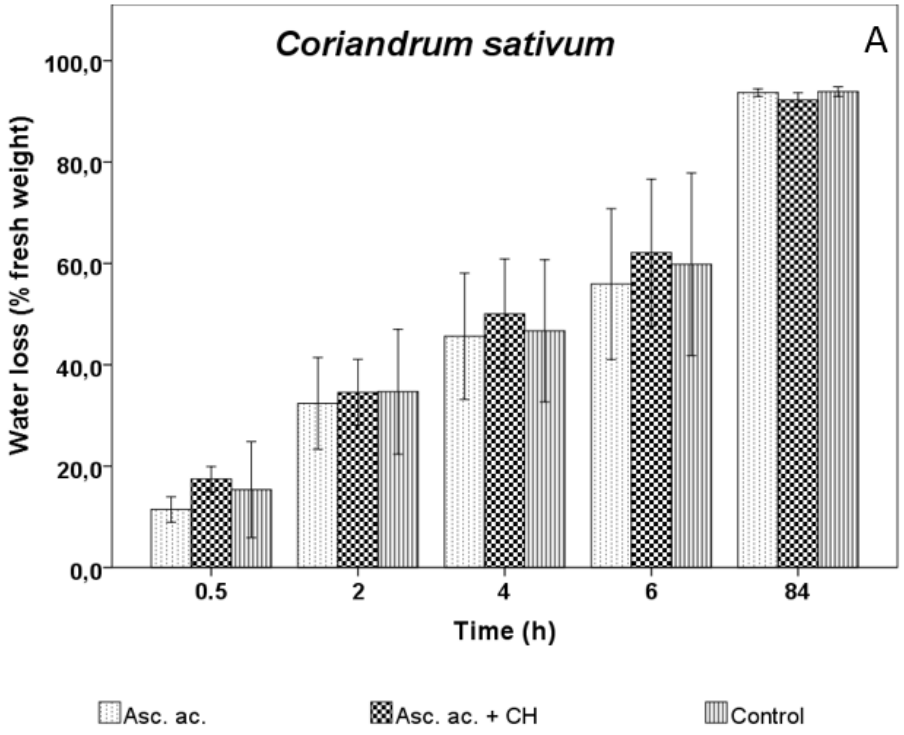
Ranil et al. (2015) evaluate the effects of potential factors involved on the enhancement of seed germination in dormant seeds of *Solanum torvum*, using an orthogonal array experimental design. The investigators evaluated soaking (water and 500 ppm solution of GA_3 for 1 day), among other conditions on the germination of *S. torvum* seeds. They concluded that soaking seeds of *S. torvum* resulted in early germination, being significantly superior to the rest of the treatment.

Figure 12 demonstrate that *S. lycopersicum* (Fig. 12B) had more efficient germination when chitosan are dissolved in ascorbic acid. In acetic acid once the germination doesn't are visualized, having zero plant germinated in 21 days. This results demonstrate that the seed are ennhibited when they are subjected to acetic acid medium during 3 hours. The maximum germination occurs in ascorbic acid, having a germination percentage in a range of 92-93%. The control have a range of 57-58% and ascorbic acid with chitosan 78-79%.

4.4.2. Water loss of detached leaves

In the short term, stomatal closure to avoid excessive water losses occurs rapidly and is widely recognized as the primary effect of drought on carbon assimilation (Chaves et al. 2002). However, avoidance of excessive water losses occurs at the expense of reducing the CO_2 availability inside the leaf, and therefore limiting photosynthesis (Galmés et al. 2007).

Under drought, stomatal size often becomes smaller (Gindel 1969; Clifford et al. 1995), and small and abundant stomata imply faster response to excessive water losses and enhance fine regulation of plant water use (Franks and Beerling 2009).



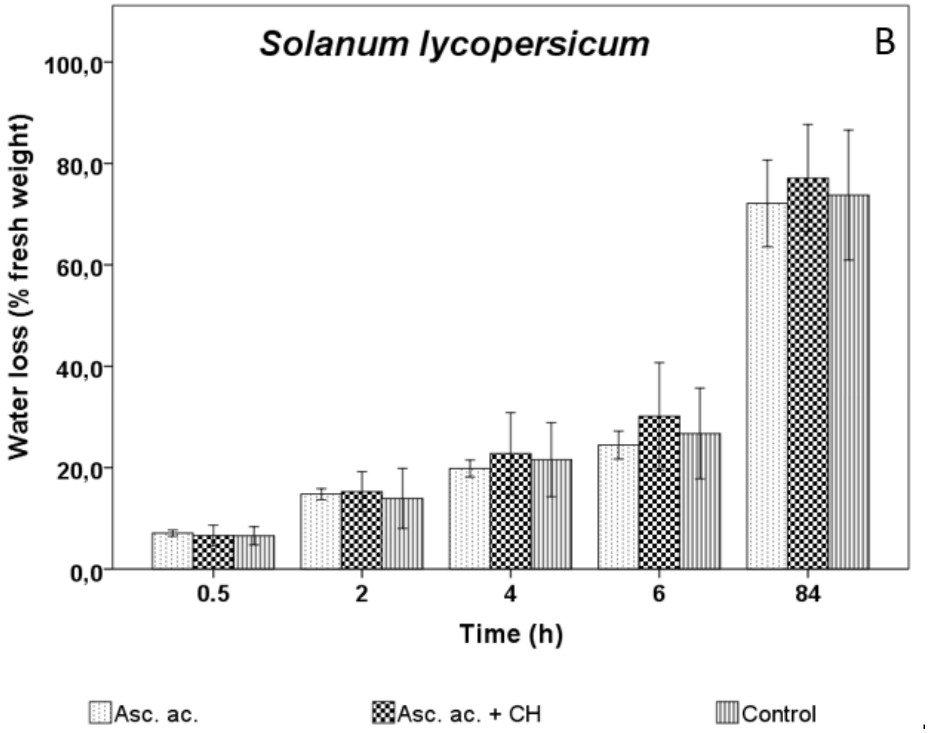


Figure 13 - Water loss by leaves of *C. sativum* (A) and *S. lycopersicum* (B) under stress condition caused by the fact of being detached from the stem and under hot conditions. Data are means ± Standard deviation (n=4). Asterisks indicate significantly different between the control and the conditions determined by Dunnett's *t* test ($0.05 \leq P$).

No statistical differences between conditions had been registered, only between species.

The percentage of water content lots by leaves (Fig. 13) are in accordance more or less in almost conditions, losing consistently their water present in leaves after 84h of experiment.

Fallow the results showed for *C. sativum* on the figure 13A after 30 min the water lost by the control condition was 15.3%, the water lost by the condition ascorbic acid with chitosan was 17.4% and the condition ascorbic acid was 11.4%. After 2h, the content of water lost by the conditions are more closely, starting to had an approach on amount of water content lost. The content of water lost was 34.6% for control condition, 34.6% for ascorbic acid with chitosan condition and 32.4% for ascorbic acid without chitosan condition. After 4h, the control had 46.7% of water lost, the ascorbic acid with chitosan 50.0% and the condition ascorbic acid without chitosan 45.6%. 6h later of the beginning of the experiment, the percentage of water lost by the control its 59.8%, the water lost by the

condition ascorbic acid with chitosan was 62.1% and the water lost by the condition ascorbic acid without chitosan was 55.9%. Regarding this last results, after 6h, for *C. sativum*, the condition that offer more resistance is the ascorbic acid without chitosan, however the drying of the leaves occurs more or less equal for each condition, didn't differentiate to much the condition that have chitosan and the controls.

Moreover, the specie *S. lycopersicum* (Fig. 13B) in ascorbic acid with chitosan condition had a percentage of 6.6% of water lost in the first half hour. After 2 hours of the beginning of the assay the water lost by the leaves was 15.3%. 4 hours later, the percentage of water lost by the leaves was 22.8%. After 6 hours it was 30.1%. At the end of the assay (84 hours) the percentage of water lost by the leaves was 77.1%. For ascorbic acid without chitosan condition, the percentage of water lost in the first half hour was 7.1%, after 2 hours was 14.8%, after 4 hours was 19.8%, after 6 hours was 24.5% and at the end of the assay was 72.1%. For control condition, the first half hour, the percentage of water lost was 6.6%, after 2 hours was 14%, after 4 hours was 21.6%, after 6 hours was 26.7% and at the end of the assay was 73.7%. Taking in account this result it's perceptible that the major percentage of water lost in leaves of *S. lycopersicum* occurs more in the condition of ascorbic acid with chitosan.

It was verified that the temperature favors the drying time decrease and drying rate increase. This behavior was described both by Doymaz (2005), when studying the drying of mint leaves, and Sharma et al. (2005), when studying the drying kinetics of onion.

4.4.3. Plant growth

Chitosan easily absorbs to epidermis of leaves and stems prolonging the contact time and facilitating the uptake of the bioactive molecules (Malerba and Cerana 2016).

González Peña et al. (2014) evaluate the effect of chitosan in *S. lycopersicum* growth. Tomato seeds were imbibed 4 hours in chitosan solutions of 0.1; 1.0; 2.5 and 10 g.L-1. Root length and stem length were evaluated in several moments. The results for the concentration of 10g/L showed that the root length is inhibited. For the stem length the plants showed normal evolution being more or less equal to control.

For root growth the control and the ascorbic acid highlight to the chitosan dissolved in ascorbic acid. Control condition reach an growth of 7.7 ± 2.44 cm and ascorbic acid reach

7.7 ± 2.08 cm. For the condition ascorbic acid with chitosan the maximum growth was 4.5 ± 1.56 cm.

C. sativum had more stem growth when ascorbic acid are present with chitosan, having an growth of 8.0 ± 1.03 cm. Ascorbic acid without chitosan had, after 21 days, 7.9 ± 0.99 cm and control had 7.5 ± 1.21 cm. Once more, the conditions doesn't variate between them.

Table 8. Root and stem length (cm) of *C. sativum* after 21 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=4). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Coriandrum sativum

	21 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	7.7 ± 2.61 $_{a}$	7.5 ± 1.29 $_{a}$	15.3 ± 2.58 $_{a}$
Asc. Ac. + CH	4.5 ± 1.66 $_{a}$	8.0 ± 1.12 $_{b}$	12.4 ± 2.29 $_{b}$
Asc. Ac.	7.7 ± 2.22 $_{b}$	7.9 ± 1.06 $_{b}$	15.6 ± 2.45 $_{b}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: $0.05 \leq p < 0.01$.

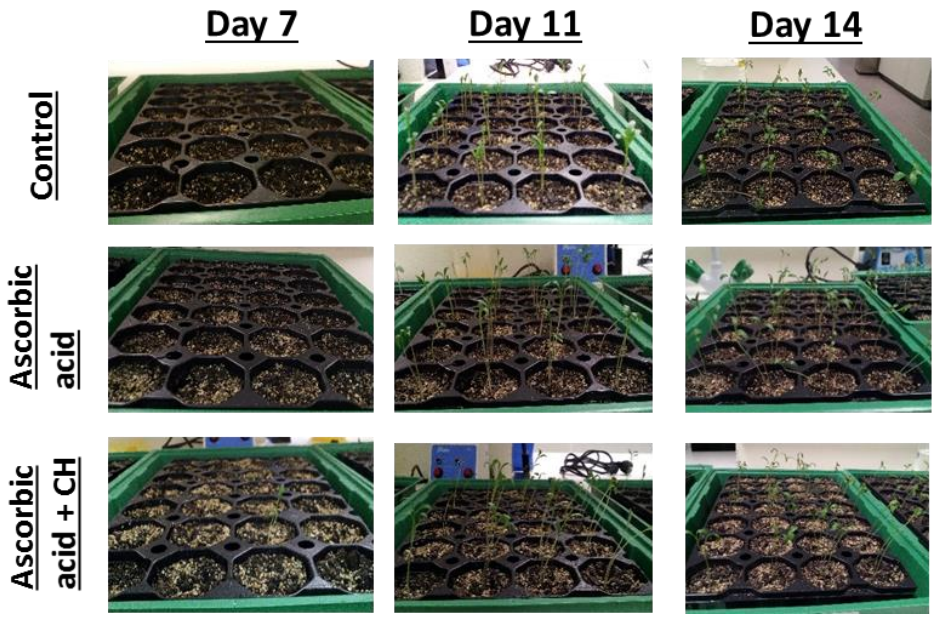


Figure 14 – Seedlings of *Coriandrum sativum* after 7, 11 and 14 days of culture.

On *C. sativum* total growth (Table 8) was observed that the condition that make plant growth more is ascorbic acid with 15.6 ± 2.29 cm. The condition ascorbic acid with chitosan showed to be less effective in terms of length, registered a total length of 12.4 ± 2.14 cm after 21 days. The control had registered a length of 15.3 ± 2.41 close for the registered by the condition ascorbic acid.

After 21 days was notable, for *S. lycopersicum* (Table 9), that control were the condition that provided more root growth (7.3 ± 1.66 cm), being the condition ascorbic acid with chitosan that provided worst results (5.3 ± 0.88 cm), comparing this three results.

Table 9. Root and stem length (cm) of *S. lycopersicum* after 21 days of seed soaking with ascorbic acid solution, acetic acid solution (Ascorbic acid and Acetic acid), chitosan (Ascorbic acid + Chitosan and Acetic acid + Chitosan) and control. Data are means ± Standard deviation (n=4). Equal superscripts in the same row are significantly different between the treatments at 5% level according to Dunnett's t-test (p≤ 0.05).

Solanum lycopersicum			
	21 days		
	Root (cm)	Stem (cm)	Total (cm)
Control	7.3 ± 1.66 $_{a}$	7.6 ± 0.55 $_{a}$	14.9 ± 1.88 $_{a}$
Asc. Ac. + CH	5.3 ± 0.88 $_{b}$	7.3 ± 1.14 $_{b}$	12.6 ± 1.65 $_{b}$
Asc. Ac.	5.7 ± 2.14 $_{b}$	7.4 ± 0.14 $_{b}$	13.1 ± 2.16 $_{b}$

$^{a \text{ and } b}$ All analyses were carried out with a significance levels by Dunnett´s multiple comparison test within treatments and control: * $0.05 \leq p < 0.01$.

For stem growth of *S. lycopersicum*, the conditions doesn't variate significantly between them, being more or less equal each other. Once the condition control (7.6 ± 0.55 cm) provide more growth, comparing the three conditions.

The total growth for the species studied didn't differ much, since after 21 days of experience, the species obtained a growth above 10 cm. For this experiment, once again, chitosan showed worse results compared to the studied conditions, showing no influence on plant growth.

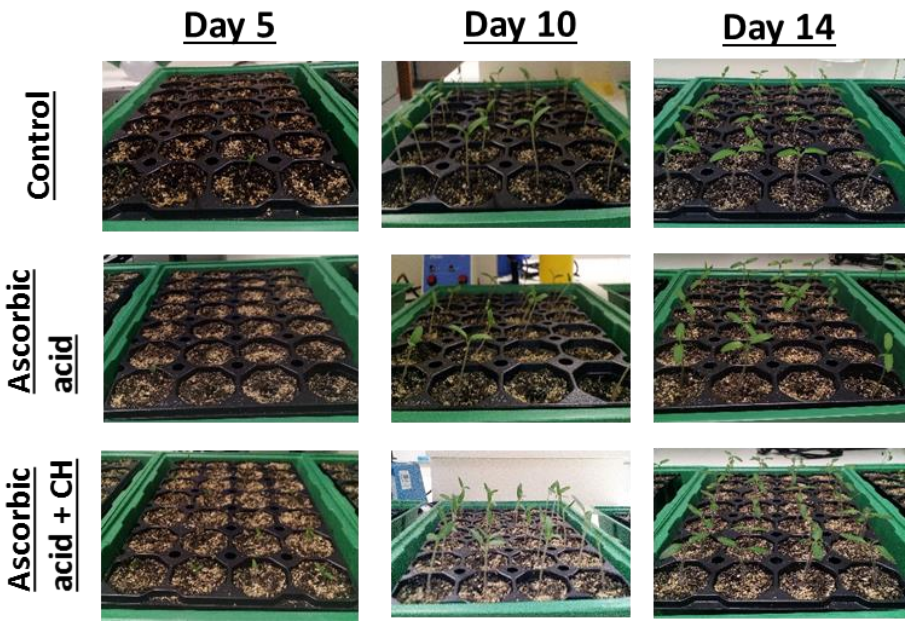


Figure 15 – Seedlings of *Solanum lycopersicum* after 5, 10 and 14 days of culture.

Several studies carried out by other authors summarize the effect of chitosan on the germination of different seeds and coincide with the results presented in the present work (Sharathchandra et al., 2004; Guan et al., 2009).

4.5. LPO

Lipid peroxides exert their toxic effects through two general mechanisms. Once the lipids are responsible for keeping the cell membrane integrity, extensive peroxidation of lipids alter the composition, assembly, structure and dynamics of lipids (Gaschler and Stockwell, 2017).

With regard to antioxidant properties assayed, the lipidperoxidation inhibition, as a vital indicator, is often used. Malondialdehyde (MDA), one of the end products of lipid peroxidation,and thiobarbituric acid (TBA) interact with each other at high temperature to form a pink compound that is detected by the spectrophotometer (Isaksson et al., 2009).

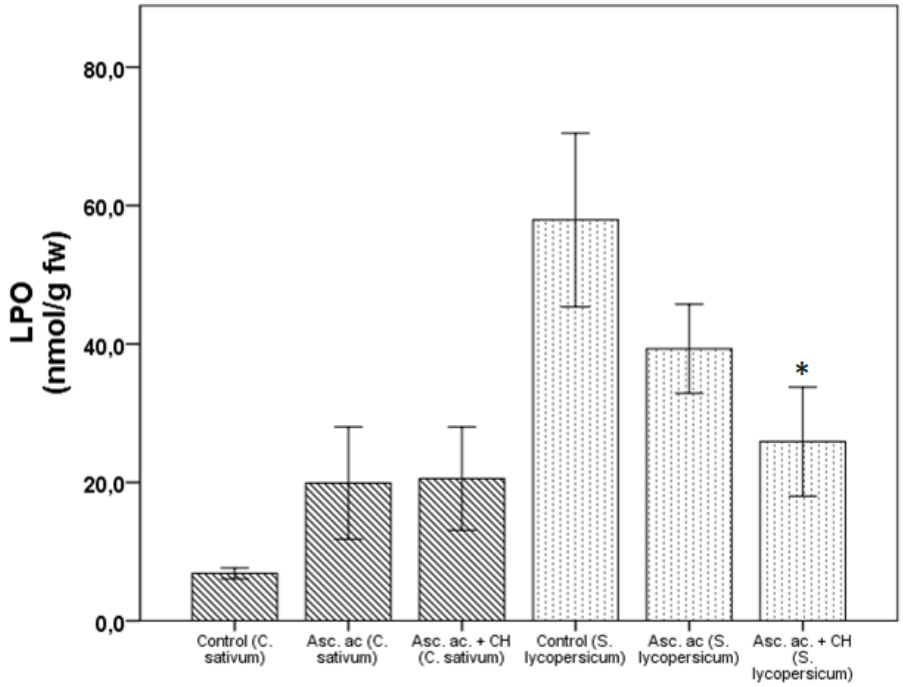


Figure 16 - Lipid peroxidation of *C. sativum* and *S. lycopersicum*. Asterisks indicate significant differences from the corresponding control values determined by Dunnett's t test (P ≤ 0.05).

The lipid peroxidation that occurs in response to stress is proportional to the levels of antioxidants and ROS-scavenging enzymes present in the plant tissues. Measurement of MDA in plant tissues can therefore be used as a valid indicator of the extent of damage occurring to plant cells in response to oxidative stress (Grotto et al. 2009).

Lipid peroxidation was further employed to see if exist differences between conditions and species. As showed in figure 16, the lipid peroxidation only showed differences between the control and ascorbic acid with chitosan of *S. lycopersicum*. Between species (*Corandrium* and *Solanum*) the differences were more understandable showing huge differences among them specialy the control of *C. sativum* and all the conditions of *S. lycopersicum*. Chitosan pretreatment effectively decreased this peroxidation, with an optimum response obtained with a 100 mg.l^{-1} concentration (P\0.05).

Few information is known regarding the effect of chitosan and its derivatives on the lipid peroxidation of plants when expose to biotic stressors.

Chapter 5: Conclusion

The present work aimed to study the effects of chitosan extracted from marine resources on biophysical characteristics of *Solanum lycopersicum* and *Coriandrum sativum*.

The lower yields of polymers isolated (chitin/chitosan) from the shrimp exoskeleton, when compared with other crustacean sources, showed that was necessary more or less 500g to produce approximately 10% of chitosan. The mineral content existent in samples are in accordance to the results of the chitosan commercialized.

Chitosan samples obtained from shrimp exoskeleton, were used to determine the polydispersity index and the viscosity molecular weight of polymer. According to Ribeiro et al., (2014) the low molecular weight have an average of approximately 120kDa. Comparing with the results obtained (Mw = 56.5 kDa) it can be said that our determined value were in a range of low molecular weight but the polydispersion index obtained indicates that the particle size distribution is spreaded being roughly related to the low purity of the polymer.

For experiment 1, can be concluded for germination rate that for *C. sativum* the plants starts to growth after 8-9 days, included the seeds that were submitted to chitosan, but after 16 days of experiment the conditions with chitosan had less percentage of plants germinated than the other conditons, being that number didn't change after 30 days. For *S. lycopersicum*, the plants start to grow more early (4-5 days) as expected and after 16 days of experiment the seeds embebed in chitosan medium had more percentage of plants development than the other conditons. The number of plants didn't change during the last days.

After 30 days of experiment we can't conclude that the chitosan had influence in retention of water on leaves tissues, being the control the result that were in accordance on the two species. For root and stem length the results doesn't showed that chitosan had influence on plant growth. Comparing the obtained results it was possible to conclude that the conditions without chitosan, obtained a greater growth from the 15th day until the 30th day of experiment.

For second experiment, can be concluded that germinaton rate for *C. sativum*, chitosan had no influence on early germinaton, once all plants "born" at same time, but for the

percentage of plants germinated chitosan reach almost 100% and chitosan possibly had influence on that. For *S. Lycopersicum*, chitosan had influence on early germinaton once after 5 days of seeds being planted, they start to growth, but after 21 days the percentage of plants growth are much less than the remaining conditons, contrary to what happened to *C. sativum*.

For water loss by leaves tissues it can be concluded that chitosan had no influence on retention of water, once all the conditons lost water gradualy on two species. In this context the simple fact of immersing the seeds in chitosan solvents may not be sufficient for water loss, and a foliar application treatment of chitosan is necessary.

For plant growth (root and stem), after 21 days we can't conclude that chitosan had no influence, once the seeds that were submited to chitosan on the beginning of the experiment had less growth than the other conditons.

Since the concentration of chitosan used was 10g.L-1 inhibition of the plants partial growth occurs, namely from the root. This fact is probably due to the toxic levels for certain germination-related events. Due to the viscosity of the polymer, which may also influence the results obtained, the formation of a film around the seed may have reduced the exchange of gases with the medium, limiting the development of plants, namely the roots the major precursor of nutrient uptake and water (Reddy et al., 2000).

The lipid peroxidation was maded to see if chitosan pretreatment decreased peroxidation caused by stress, with an optimum response obtained with a 100 $mg.l^{-1}$ concentration.

In conclusion, the characteristics of this non-tradiotional marine resource and its biological properties, support the hypothesis of using it as raw material for biotechnology industries enhancing by that way its economic value.

The promotion of this bycatch specie, also increases competitiveness on fishing activities through reorientation of fisheries and diversification of targeted species for biotechnology purposes, with potential implication on the sustainable development of coastal communities.

Chapter 6: Future Perspectives

Through this study, same disadvantages were noted and keeping the records of those for in the future make some differences and trying improve them.

The first disadvantages noted were on the transformation of the raw material. First, the costs to transform by chemical processes are expensive. Secondly the time to obtain only 10g of chitosan is too much. Third, the homogeneity in the last step didn't were resolute, increasing the disparities between temperatures inside the flask and produces polymers with different biochemical/structural properties.

In order to improve, some aspects have to change. First in terms of raw material transformation costs, the reuse of solvents was the first propose given, but the viscosity obtained at the end of all steps make the reuse of solvents impossible. The components distillation with different volatilities it's the better propose, dividing the solvent that are able to reuse and the discarded solvent with unnecessary components dissolved from each step.

Given answer for the second disadvantage noted, the use of bacterias and enzymes, one approach more eco-friendly and in turn a biological extraction chitosan, it's a better propose, once the time expended will reduce given the velocity of bacterias and enzymes will degrade the components not used.

At least, for a better homogeneity in the last step (deacetylation), the use of a closed bioreactor could give a total flux in all material present, submitting all material at same conditions.

For future proposes:

(1) Using different concentrations of chitosan (0,1; 0,5; 1,0; 1,5; 2 $g.L^{-1}$) and different times of soaking (3h; 6h; 9h) and register what concentration in what time have more beneficial effects on germination and plant growth in Solanum and Coriandrum species

(2) Using different types of priming/soaking (hydropriming, halopriming, osmopriming and hormonal priming) with solutions/polymers profitables and see what they are

better in terms of germination percentage, fruits development, etc. This propose could be a huge advantage for the economical approach in agriculture, reducing the costs used.

(3) In disadvantages conditions (acid soils, salt soils, metal soil, high and low temperatures, etc) see if the different priming/soaking have results.

(4) Using more quantity of leaves to do LPO.

Chapter 7: References

Aguirre GA (2001). *Química de los suelos ácidos, templados y tropicales* (No. 631.41 A3QU5).

Ahmed, A. B. A., & Kim, S. K. (2010). Chitin, Chitosan Derivatives Induce the Production of Secondary Metabolites and Plant Development through In Vitro and In Vivo Techniques. In *Chitin, Chitosan, Oligosaccharides and Their Derivatives: Biological Activities and Applications* (pp. 589-603). CRC Press.

Albersheim, P. & Darvill, A. G. (1985). “Oligosaccharins” Scientific American, vol. 253, pp. 58–64, 1985.

Alscher, R. G., Donahue, J. L., & Cramer, C. L. (1997). Reactive oxygen species and antioxidants: relationships in green cells. *Physiologia Plantarum*, *100*(2), 224-233.

Alscher, R.G.; Erturk, N. & Heath, L.S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. Journal of Experimental Botany, Vol.53, No. 372. pp. 1331-1341.

Altieri, M. A., & Nicholls, C. I. (2003). Soil fertility management and insect pests: harmonizing soil and plant health in agroecosystems. *Soil and Tillage Research*, *72*(2), 203-211.

Alves, N. M., & Mano, J. F. (2008). Chitosan derivatives obtained by chemical modifications for biomedical and environmental applications. *International journal of biological macromolecules*, *43*(5), 401-414.

Amer, M. A., Hoorne, D., & Poppe, J. (1993). In-vivo evaluation of adjuvants for more effective control of celery leaf-spot (Septoria apiicola) and powdery mildew (Erysiphe graminis) of wheat with fungicides. *Pesticide science*, *37*(2), 113-120.

Andreoli, C., & Khan, A. A. (1997, October). Integration of physiological, chemical, and biological seed treatments to improve stand establishment and yield of vegetables.

Anon. (2008). The UK Pesticide Guide. British Crop Protection Council. Cabi Publishing, Oxon, UK.

Apel, K., & Hirt, H. (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.*, *55*, 373-399.

Ashraf, M. and Rauf, H. (2001). Inducing salt tolerance in maize (Zea mays L.) through seed priming with chloride salts: Growth and ion transport at early growth stages. Acta Physiol Pl. 23407–414.

Ashraf, M., Akram, N. A., Arteca, R. N., & Foolad, M. R. (2010). The physiological, biochemical and molecular roles of brassinosteroids and salicylic acid in plant processes and salt tolerance. *Critical Reviews in Plant Sciences*, *29*(3), 162-190.

Assis, O. B. G. (2008). Avaliação, por análise de imagens, da ação fungistática de coberturas de quitosana em maçãs minimamente processadas. *Monografia de Especialização, Universidade Federal de Lavras, Brasil*.

Azadi, H., Schoonbeek, S., Mahmoudi, H., Derudder, B., De Maeyer, P., & Witlox, F. (2011). Organic agriculture and sustainable food production system: main potentials. *Agriculture, Ecosystems & Environment*, *144*(1), 92-94.

Backman, P. A. (1978). Fungicide formulation: relationship to biological activity. *Annual Review of Phytopathology*, *16*(1), 211-237.

Badawy, M. E., & Rabea, E. I. (2011). A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. International Journal of Carbohydrate Chemistry, 2011.

Badgujar, C. D., & Warhal, K. N. (1988). Effect of seed soaking and wrapping on growth and yield of vegetable coriander. J. Maharashtra Agricultural Univ, 13(3), 344-65.

Bailey-Serres, J., & Mittler, R. (2006). The roles of reactive oxygen species in plant cells.

Banafar, R. N. S. (1994). Influence of hormones and Chemicals on the germination of coriander seed (Coriandrum sativum L.). Gujarat Agricultural University Research Journal, 20, 111-113.

Bautista-Baños, S., Hernández López, M., & Bosquez Molina, E. (2004). Growth inhibition of selected fungi by chitosan and plant extracts. *Revista Mexicana de fitopatología*, *22*(2).

Bautista-Baños, S., Hernández-López, M., Bosquez-Molina, E., & Wilson, C. L. (2003). Effects of chitosan and plant extracts on growth of Colletotrichum gloeosporioides, anthracnose levels and quality of papaya fruit. *Crop protection*, *22*(9), 1087-1092.

Benhamou, N., & Lafontaine, P. J. (1995). Ultrastructural and cytochemical characterization of elicitor-induced structural responses in tomato root tissues infected by Fusarium oxysporum f. sp. radicis-lycopersici. *Planta*, 89-102.

Bennett, A. J., Bending, G. D., Chandler, D., Hilton, S., & Mills, P. (2012). Meeting the demand for crop production: the challenge of yield decline in crops grown in short rotations. *Biological Reviews*, *87*(1), 52-71.

Bewley, J.D., Bradford, K., Hilhorst, H., Nonogaki, H. (2013). Seeds: Physiology of Development, Germination and Dormancy, 3rd edition. Springer, New York.

Bittelli, M., Flury, M., Campbell, G. S., & Nichols, E. J. (2001). Reduction of transpiration through foliar application of chitosan. *Agricultural and Forest Meteorology*, *107*(3), 167-175.

Blaedow, R. A., Chaney, W. R., Pecknold, P. C., & Holt, H. A. (2006). Investigation of fungicidal properties of the tree growth regulator paclobutrazol to control apple scab. JOURNAL OF ARBORICULTURE, 32(2), 67.

Blankendaal, M. (1972). Growing plants without soil for experimental use (No. 1251). Agricultural Research Service, US Department of Agriculture.

Boyer, J. S., & Westgate, M. E. (2004). Grain yields with limited water. *Journal of experimental Botany*, *55*(407), 2385-2394.

Brand, U., Hobe, M., & Simon, R. (2001). Functional domains in plant shoot meristems. *Bioessays*, *23*(2), 134-141.

Bukrudeen, A., Ahmed, A., Kim, S.W. (2010). Chitin and chitosan derivatives induce the production of secondary metabolites and plant development through in vitro and in vivo technique. In: Kim, Se Kwon (Ed.), Chitin, Chitosan, Oligosaccharides and their Derivatives: Biological Activity and Application. CRC Press, Taylor & Francis, pp. 589–603.

Bumgardner, J. D., Wiser, R., Gerard, P. D., Bergin, P., Chestnutt, B., Marini, M., ... & Gilbert, J. A. (2003). Chitosan: potential use as a bioactive coating for orthopaedic and craniofacial/dental implants. *Journal of Biomaterials Science, Polymer Edition*, *14*(5), 423-438.

Bunting, J. A., Sprague, C. L., & Riechers, D. E. (2004). Proper adjuvant selection for foramsulfuron activity. *Crop Protection*, *23*(4), 361-366.

Capanoglu, E. (2010). The potential of priming in food production. *Trends in food science & technology*, *21*(8), 399-407.

Carson, R. L. (1962). Silent Spring, Riverside Press. *Cambridge, MA, USA*.

Carter, L. M., & Chesson, J. H. (1996). Two USDA researchers develop a moisture seeking attachment for crop seeders that is designed to help growers plant seed in soil sufficiently moist for germination. *Seed World*, *134*, 14-15.

Casida, J. E., & Quistad, G. B. (1998). Golden age of insecticide research: past, present, or future?. *Annual review of entomology*, *43*(1), 1-16.

Chandrkrachang, S. (2002). The application of chitin and chitosan in agriculture in Thailand. *Advances in chitin science*, *5*(1), 458-462.

Chau, V. M., Pham, H. D., Dang, L. H., Trinh, D. H., and Hoang, T. H (1996). “Use of chitosan in agriculture and food preservation. I. Chitosan as a fresh fruit pres,” Vietnam Journal of Chemistry, vol. 34, pp. 29–33.

Chaves, M. M., Pereira, J. S., Maroco, J., Rodrigues, M. L., Ricardo, C. P. P., Osório, M. L., ... & Pinheiro, C. (2002). How plants cope with water stress in the field? Photosynthesis and growth. *Annals of botany*, *89*(7), 907-916.

Cheah, L. H., Page, B. B. C., & Shepherd, R. (1997). Chitosan coating for inhibition of sclerotinia rot of carrots.

Chibu, H., Shibayama, H. (2000). Effects of chitosan application on the growth of several crops. In: Uragami, T., Kurita, K., Fukamizo, T. (Eds.), Chitin Chitosan in Life Science. Kodansha Scientific LTD, Tokyo, pp. 235–239.

Chirkov, S. N. (2002). The antiviral activity of chitosan (review). *Applied Biochemistry and Microbiology*, *38*(1), 1-8.

Chmielewski, A. G., Migdal, W., Swietoslawski, J., Jakubaszek, U., & Tarnowski, T. (2007). Chemical-radiation degradation of natural oligoamino-polysaccharides for agricultural application. *Radiation Physics and Chemistry*, *76*(11), 1840-1842.

Clasen, C., Wilhelms, T., & Kulicke, W. M. (2006). Formation and characterization of chitosan membranes. *Biomacromolecules*, *7*(11), 3210-3222.

Clifford, S. C., Black, C. R., Roberts, J. A., Stronach, I. M., Singleton-Jones, P. R., Mohamed, A. D., & Azam-Ali, S. N. (1995). The effect of elevated atmospheric CO2 and drought onstomatal frequency in groundnut (Arachis hypogaea (L.)). *Journal of Experimental Botany*, *46*(7), 847-852.

Conklin, P. L., Williams, E. H., & Last, R. L. (1996). Environmental stress sensitivity of an ascorbic acid-deficient Arabidopsis mutant. *Proceedings of the National Academy of Sciences*, *93*(18), 9970-9974.

Creissen, G., Firmin, J., Fryer, M., Kular, B., Leyland, N., Reynolds, H., ... & Mullineaux, P. (1999). Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco plants paradoxically causes increased oxidative stress. *The Plant Cell*, *11*(7), 1277-1291.

Cui, Z., Yue, S., Wang, G., Zhang, F., & Chen, X. (2013). In-season root-zone N management for mitigating greenhouse gas emission and reactive N losses in intensive wheat production. *Environmental science & technology*, *47*(11), 6015-6022.

Dąbrowska, B., Capecka, E. and Suchorska, K. (1999). Results of presowing seed priming of coriander (Coriandrum sativum L.). Umbell. Improv. Newslett. 9: 27-29.

Dalton, T. P., Shertzer, H. G., & Puga, A. (1999). Regulation of gene expression by reactive oxygen. *Annual review of pharmacology and toxicology*, *39*(1), 67-101.

Darvill, A., Augur, C., Bergmann, C., Carlson, R. W., Cheong, J. J., Eberhard, S., ... & Mohnen, D. (1992). Oligosaccharins—oligosaccharides that regulate growth, development and defence responses in plants. *Glycobiology*, *2*(3), 181-198.

Davies, B., Baulcombe, D., Crute, I., Dunwell, J., Gale, M., Jones, J., ... & Toulmin, C. (2009). Reaping the benefits: science and the sustainable intensification of global agriculture.

De Rutter, H., Uffing, A. J., Meinen, E., & Prins, A. (1990). Influence of surfactants and plant species on leaf retention of spray solutions. *Weed Science*, 567-572.

Dodds, D. M., Reynolds, D. B., Massey, J. H., Smith, M. C., & Koger, C. H. (2007). Effect of adjuvant and urea ammonium nitrate on bispyribac efficacy, absorption, and translocation in barnyardgrass (Echinochloa crus-galli). II. Absorption and translocation. *Weed science*, *55*(5), 406-411.

Doymaz, I. (2004). Effect of pre-treatments using potassium metabisulphide and alkaline ethyl oleate on the drying kinetics of apricots. *Biosystems Engineering*, *89*(3), 281-287.

Dutta, P. K., Dutta, J., & Tripathi, V. S. (2004). Chitin and chitosan: Chemistry, properties and applications.

Dzung, N. A. (2007). Chitosan and their derivatives as prospective biosubstances for developing sustainable eco-agriculture. *Advances in chitin science X*, 453-459.

Dzung, N. A. (2010). Enhancing crop production with chitosan and its derivatives. In *Chitin, chitosan, oligosaccharides and their derivatives: biological activities and applications* (pp. 619-631). CRC Press.

Dzung, N. A., & Thang, N. T. (2002). Effects of chitooligosaccharides prepared by enzyme degradation on the growth of soy bean. *Advances in Chitin Science*, *463*, 467.

Dzung, N. A., & Thang, N. T. (2004). Effect of chitooligosaccharides on the growth and development of peanut (Arachis hypogea L.). In *Proceedings of the Sixth Asia-Pacific on Chitin, Chitosan Symposium.(Ed.) E. Khor, D. Hutmacher and LL Yong. Singapore, isbn* (pp. 981-05).

Dzung, N. A., & Thuoc, V. (2006). Study on effects of chitooligosaccharides on the growth development and disease resistance of rice. *Advances in Chitin Science and Technology. Hanrimwon Seoul, ISBN*, 89-5708.

Dzung, N. A., Khanh, V. T. P., & Dzung, T. T. (2011). Research on impact of chitosan oligomers on biophysical characteristics, growth, development and drought resistance of coffee. *Carbohydrate polymers*, *84*(2), 751-755.

Dzung, N. A., Khanh, V. T. P., & Ngoc, N. T. B. (2006). Study on effects of chitosan, chitosan oligomer and their derivatives on the growth inhibition of pathogenous fungi Phytopthora capsici and application for biocontrol black pepper. *Advances in chitin science and technology*, 505-507.

Dzung, N.A. (2005). Application of chitin, chitosan and their derivatives for agriculture in Vietnam. J. Chitin Chitosan 10 (3), 109–113.

El Ghaouth, A., Arul, J., Asselin, A., & Benhamou, N. (1992). Antifungal activity of chitosan on post-harvest pathogens: induction of morphological and cytological alterations in Rhizopus stolonifer. *Mycological research*, *96*(9), 769-779.

El Hadrami, A., Adam, L. R., El Hadrami, I., & Daayf, F. (2010). Chitosan in plant protection. Marine drugs, 8(4), 968-987.

El-Sawy, N. M., El-Rehim, H. A. A., Elbarbary, A. M., & Hegazy, E. S. A. (2010). Radiation-induced degradation of chitosan for possible use as a growth promoter in agricultural purposes. *Carbohydrate polymers*, *79*(3), 555-562.

Elstner, E. F. (1991). Mechanisms of oxygen activation in different compartments of plant cells. *Current topics in plant physiology (USA)*.

Faoro, F., Sant, S., Iriti, M., Maffi, D., & Appiano, A. (2001). Chitosan-elicited resistance to plant viruses: a histochemical and cytochemical study. *Chitin enzymology*, *22*, 57-62.

Ferrari, S. (2010). Biological elicitors of plant secondary metabolites: mode of action and use in the production of nutraceutics. In *Bio-Farms for Nutraceuticals* (pp. 152-166). Springer US.

Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental botany*, *55*(396), 307-319.

Foryer, C. H., & Noctor, G. (2000). Oxygen processing in photosynthesis: regulation and signaling. *New Phytol*, *146*, 359-388.

Franks, P. J., & Beerling, D. J. (2009). Maximum leaf conductance driven by CO2 effects on stomatal size and density over geologic time. *Proceedings of the National Academy of Sciences*, *106*(25), 10343-10347.

Frost, C. J., Mescher, M. C., Carlson, J. E., & De Moraes, C. M. (2008). Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, *146*(3), 818-824.

Fuller, M. P., Hamed, F., Wisniewski, M., & Glenn, D. M. (2003). Protection of plants from frost using hydrophobic particle film and acrylic polymer. *Annals of applied biology*, *143*(1), 93-98.

Gale, J., & Hagan, R. M. (1966). Plant antitranspirants. *Annual Review of Plant Physiology*, *17*(1), 269-282.

Galmés, J., Medrano, H., & Flexas, J. (2007). Photosynthetic limitations in response to water stress and recovery in Mediterranean plants with different growth forms. *New Phytologist*, *175*(1), 81-93.

Gaschler, M. M., & Stockwell, B. R. (2017). Lipid peroxidation in cell death. *Biochemical and biophysical research communications*, *482*(3), 419-425.

Ghasemnezhad, M., & Shiri, M. A. (2010). Effect of chitosan coatings on some quality indices of apricot (Prunus armeniaca L.) during cold storage. *Caspian Journal of Environmental Sciences*, *8*(1), 25-33.

Ghasemnezhad, M., Nezhad, M. A., & Gerailoo, S. (2011). Changes in postharvest quality of loquat (Eriobotrya japonica) fruits influenced by chitosan. *Horticulture, Environment, and Biotechnology*, *52*(1), 40-45.

Gindel, I. (1969). Stomatal number and size as related to soil moisture in tree xerophytes in Israel. *Ecology*, *50*(2), 263-267.

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and planetary change*, *63*(2), 90-104.

Girotti, A. W. (2001). Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *Journal of Photochemistry and Photobiology B: Biology*, *63*(1), 103-113.

González Peña, D., Costales, D., & Falcón, A. B. (2014). Influence of a chitosan polymer in the growth and the induction of defensive enzymes in tomato (Solanum lycopersicum L.). Cultivos Tropicales, 35(1), 35-42.

Gratão, P. L., Polle, A., Lea, P. J., & Azevedo, R. A. (2005). Making the life of heavy metal-stressed plants a little easier. *Functional Plant Biology*, *32*(6), 481-494.

Green, J. M., Brown, P. A., Berengut, D., & King, M. G. (1992). Nonionic surfactant property effects on thifensulfuron methyl performance in soybeans.

Grether-Beck, S., Bonizzi, G., Schmitt-Brenden, H., Felsner, I., Timmer, A., Sies, H., ... & Krutmann, J. (2000). Non-enzymatic triggering of the ceramide signalling cascade by solar UVA radiation. *The EMBO journal*, *19*(21), 5793-5800.

Grotto, D., Maria, L. S., Valentini, J., Paniz, C., Schmitt, G., Garcia, S. C., ... & Farina, M. (2009). Importance of the lipid peroxidation biomarkers and methodological aspects for malondialdehyde quantification. *Quimica Nova*, *32*(1), 169-174.

Guan, Y. J., Hu, J., Wang, X. J., & 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.

Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., & Li, P. (2008). The influence of molecular weight of quaternized chitosan on antifungal activity. *Carbohydrate Polymers*, *71*(4), 694-697.

Guo, Z., Xing, R., Liu, S., Zhong, Z., Ji, X., Wang, L., & Li, P. (2007). Antifungal properties of Schiff bases of chitosan, N-substituted chitosan and quaternized chitosan. *Carbohydrate research*, *342*(10), 1329-1332.

Hadwiger, L.A. (2013). Multiple effects of chitosan on plant systems: Solid science or hype. Plant Sci. 208, 42–49.

Hall, K. J., Holloway, P. J., & Stock, D. (1997a). Factors affecting the efficiency of spray delivery onto foliage using oil-based adjuvants. *Aspects of Applied Biology*.

Hall, K. J., Holloway, P. J., & Stock, D. (1998). Factors affecting foliar retention of some model adjuvant oil-in-water emulsions. *Pest Management Science*, *54*(3), 320-322.

Hall, K. J., Western, N. M., Holloway, P. J., & Stock, D. (1997b). Effects of adjuvant oil emulsions on foliar retention and spray quality. volume 2.

Han, J. S. (1990). Use of antitranspirant epidermal coatings for plant protection in China. *Plant Disease*, *74*(4), 263-266.

Harte, J., Saleska, S., & Shih, T. (2006). Shifts in plant dominance control carbon-cycle responses to experimental warming and widespread drought. *Environmental Research Letters*, *1*(1), 014001.

Hartzler, R. G., & Foy, C. L. (1983). Efficacy of three postemergence grass herbicides for soybeans. *Weed Science*, 557-561.

Hawkesworth, S., Dangour, A. D., Johnston, D., Lock, K., Poole, N., Rushton, J., ... & Waage, J. (2010). Feeding the world healthily: the challenge of measuring the effects of

agriculture on health. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, *365*(1554), 3083-3097.

Hayati, N. E., Sukprakarn, S., & Juntakool, S. (2005). Seed germination enhancement in Solanum stramonifolium and Solanum torvum. Kasetsart J.(Nat. Sci.), 39, 368-376.

Holloway, P. J. (1994). Physicochemical factors influencing the adjuvant-enhanced spray deposition and coverage of foliage-applied agrochemicals. In *Interactions between adjuvants, agrochemicals and target organisms* (pp. 83-106). Springer Berlin Heidelberg.

Holopainen, J. K., Heijari, J., Nerg, A. M., Vuorinen, M., & Kainulainen, P. (2009). Potential for the use of exogenous chemical elicitors in disease and insect pest management of conifer seedling production.

Horst, M. N., Walker, A. N., & Klar, E. (1993). The pathway of crustacean chitin synthesis. *The crustacean integument morphology and biochemistry*, 113-149.

Houeto, P., Bindoula, G., & Hoffman, J. R. (1995). Ethylenebisdithiocarbamates and ethylenethiourea: possible human health hazards. *Environmental health perspectives*, *103*(6), 568.

Houssen, W. E., & Jaspars, M. (2012). Isolation of marine natural products. *Natural Products Isolation*, 367-392.

Hoyle, E. R., Hayes, A. L., & Holloway, P. J. (1998). Some structure-adjuvancy relationships for alkyl polyglycosides. In *Proceedings of the Fifth International Symposium on Adjuvants for Agrochemicals* (Vol. 1, p. 67).

Hunsche, M., Bringe, K., Schmitz-Eiberger, M., & Noga, G. (2006). Leaf surface characteristics of apple seedlings, bean seedlings and kohlrabi plants and their impact on the retention and rainfastness of mancozeb. *Pest management science*, *62*(9), 839-847.

Iriti, M., Picchi, V., Rossoni, M., Gomarasca, S., Ludwig, N., Gargano, M., & Faoro, F. (2009). Chitosan antitranspirant activity is due to abscisic acid-dependent stomatal closure. *Environmental and Experimental Botany*, *66*(3), 493-500.

Isaksson, C., Sturve, J., Almroth, B. C., & Andersson, S. (2009). The impact of urban environment on oxidative damage (TBARS) and antioxidant systems in lungs and liver of great tits, Parus major. *Environmental research*, *109*(1), 46-50.

Jahangir, M., Abdel-Farid, I. B., Kim, H. K., Choi, Y. H., & Verpoorte, R. (2009). Healthy and unhealthy plants: The effect of stress on the metabolism of Brassicaceae. *Environmental and Experimental Botany*, *67*(1), 23-33.

Jeong, G. T., & Park, D. H. (2005). Enhancement of growth and secondary metabolite biosynthesis: Effect of elicitors derived from plants and insects. *Biotechnology and Bioprocess Engineering*, *10*(1), 73-77.

Jing, Y. J.; Hao, Y. J.; Qu, H.; Shan, Y.; Li, D. S. & Du, R. Q. (2007). Acta Biologica Hungarica, 58, p.75-86.

Ju, X. T., Kou, C. L., Zhang, F. S., & Christie, P. (2006). Nitrogen balance and groundwater nitrate contamination: comparison among three intensive cropping systems on the North China Plain. *Environmental Pollution*, *143*(1), 117-125.

Ju, X. T., Xing, G. X., Chen, X. P., Zhang, S. L., Zhang, L. J., Liu, X. J., ... & Zhang, F. S. (2009). Reducing environmental risk by improving N management in intensive Chinese agricultural systems. *Proceedings of the National Academy of Sciences*, *106*(9), 3041-3046.

Jung, B. O., Chung, S. J., & Lee, G. W. (2002). Effect of molecular weight of chitosan on its antimicrobial activity. *Journal of Chitin and Chitosan*, *7*(4), 231-236.

Jung, W. J., & Park, R. D. (2014). Bioproduction of chitooligosaccharides: present and perspectives. *Marine drugs*, *12*(11), 5328-5356.

Kadiri, M. and Hussaini, M. A. (1999). Effect of hardening pre-treatment on vegetative growth, enzyme activities and yield of Pennisetum americanum and sorghum bicolour L. Global J Pure Appl Sci. 5: 179-183.

Kenawy, E. R., Abdel-Hay, F. I., El-Magd, A. A., & Mahmoud, Y. (2005). Biologically active polymers: Modification and anti-microbial activity of chitosan derivatives. *Journal of bioactive and compatible polymers*, *20*(1), 95-111.

Khan, A. A. (1992). Preplant physiological seed conditioning. *Horticultural reviews*, *13*(13), 131-181.

Khan, H. A., Ayub, C. M., Pervez, M. A., Bilal, R. M., Shahid, M. A. and Ziaf, K. (2009). Effect of seed priming with NaCl on salinity tolerance of hot pepper (Capsicum annuum L.) at seedling stage. Soil Environ. 28: 81-87.

Khan, W. M., Prithiviraj, B., & Smith, D. L. (2002). Effect of foliar application of chitin and chitosan oligosaccharides on photosynthesis of maize and soybean. *Photosynthetica*, *40*(4), 621-624.

Kim, H. J., Chen, F., Wang, X., & Rajapakse, N. C. (2005). Effect of chitosan on the biological properties of sweet basil (Ocimum basilicum L.). *Journal of agricultural and food chemistry*, *53*(9), 3696-3701.

Kim, H. J., Chen, F., Wang, X., & Rajapakse, N. C. (2006). Effect of methyl jasmonate on secondary metabolites of sweet basil (Ocimum basilicum L.). *Journal of Agricultural and Food Chemistry*, *54*(6), 2327-2332.

Kim, I. Y., Seo, S. J., Moon, H. S., Yoo, M. K., Park, I. Y., Kim, B. C., & Cho, C. S. (2008). Chitosan and its derivatives for tissue engineering applications. *Biotechnology advances*, *26*(1), 1-21.

Kolpin, D. W., Thurman, E. M., & Linhart, S. M. (1998). The environmental occurrence of herbicides: the importance of degradates in ground water. *Archives of Environmental Contamination and Toxicology*, *35*(3), 385-390.

Kowalski, B., Terry, F. J., Herrera, L., & Peñalver, D. A. (2006). Application of soluble chitosan in vitro and in the greenhouse to increase yield and seed quality of potato minitubers. *Potato Research*, *49*(3), 167-176.

Kraemer, T., Hunsche, M., & Noga, G. (2009). Surfactant-induced deposit structures in relation to the biological efficacy of glyphosate on easy-and difficult-to-wet weed species. *Pest management science*, *65*(8), 844-850.

Kubota, N., Tatsumoto, N., Sano, T., & Toya, K. (2000). A simple preparation of half N-acetylated chitosan highly soluble in water and aqueous organic solvents. *Carbohydrate Research*, *324*(4), 268-274.

Kudsk, P., & Mathiassen, S. K. (2007). Analysis of adjuvant effects and their interactions with variable application parameters. *Crop Protection*, *26*(3), 328-334.

Kulikov, S. N., Chirkov, S. N., Il'ina, A. V., Lopatin, S. A., & Varlamov, V. P. (2006). Effect of the molecular weight of chitosan on its antiviral activity in plants. *Applied Biochemistry and Microbiology*, *42*(2), 200-203.

Kumaresapillai, N., Basha, R. A., & Sathish, R. (2011). Production and evaluation of chitosan from Aspergillus niger MTCC Strains. *Iranian Journal of Pharmaceutical Research*, 553-558.

Lafitte, H. (2001). Estreses abióticos que afectan el maíz. *El Maíz en los Trópicos: Mejoramiento y Producción. FAO, Roma. http://www. fao. org/docrep/003/X7650S (consulta del 14/12/2011)*.

Lemarchand, C., Couvreur, P., Vauthier, C., Costantini, D., & Gref, R. (2003). Study of emulsion stabilization by graft copolymers using the optical analyzer Turbiscan. *International journal of pharmaceutics*, *254*(1), 77-82.

Lerouge, P., Roche, P., Faucher, C., Maillet, F., & Truchet, G. (1990). Symbiotic host-specificity of Rhizobium meliloti is determined by a sulphated and acylated glucosamine oligosaccharide signal. *Nature*, *344*(6268), 781.

Li, B., Shi, Y., Shan, C., Zhou, Q., Ibrahim, M., Wang, Y., ... & Sun, G. (2013). Effect of chitosan solution on the inhibition of Acidovorax citrulli causing bacterial fruit blotch of watermelon. *Journal of the Science of Food and Agriculture*, *93*(5), 1010-1015.

Li, C.F., Wu, J.C., (1998). Chitosan benefits cultivation of vegetables. In: Chen, R.H., Chen, H.C. (Eds.), Advances in Chitin Science III. Rita advertising Co. Ltd., pp. 448–451.

Li, H., & Yu, T. (2001). Effect of chitosan on incidence of brown rot, quality and physiological attributes of postharvest peach fruit. *Journal of the Science of Food and Agriculture*, *81*(2), 269-274.

Li, Y.C., Tao, W.Y. (2009). Paclitaxel-producing fungal endophyte stimulates the accumulation of taxoids in suspension cultures of Taxus cuspidate. Sci. Hort. 121, 97–102.

Limpanavech, P., Chaiyasuta, S., Vongpromek, R., Pichyangkura, R., Khunwasi, C., Chadchawan, S., ... & Bangyeekhun, T. (2008). Chitosan effects on floral production, gene expression, and anatomical changes in the Dendrobium orchid. *Scientia Horticulturae*, *116*(1), 65-72.

Lin, J. M., & Sung, J. M. (2001). Pre-sowing treatments for improving emergence of bitter gourd seedlings under optimal and sub-optimal temperatures. *Seed Science and Technology*, *29*(1), 39-50.

Lizárraga-Paulín, E. G., Torres-Pacheco, I., Moreno-Martínez, E., & 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.

López-García, J., Lehocký, M., Humpolíček, P., & Sáha, P. (2014). HaCaT keratinocytes response on antimicrobial atelocollagen substrates: extent of cytotoxicity, cell viability and proliferation. *Journal of functional biomaterials*, *5*(2), 43-57.

Luan, L. Q., Ha, V. T., Nagasawa, N., Kume, T., Yoshii, F., & Nakanishi, T. M. (2005). Biological effect of irradiated chitosan on plants in vitro. *Biotechnology and applied biochemistry*, *41*(1), 49-57.

Luan, L. Q., Nagasawa, N., Tamada, M., & Nakanishi, T. M. (2006). Enhancement of plant growth activity of irradiated chitosan by molecular weight fractionation. *Radioisotopes*, *55*(1), 21.

Magnavaca, R., & Bahia Filho, A. F. C. (1993). Success in maize acid soil tolerance. WORKSHOP ON ADAPTION OF PLANTS TO SOIL STRESSES, 1993, Lincoln. proceedings.... Lincoln: University of Nebrasca.

Malan, C., Greyling, M. M., & Gressel, J. (1990). Correlation between CuZn superoxide dismutase and glutathione reductase, and environmental and xenobiotic stress tolerance in maize inbreds. *Plant Science*, *69*(2), 157-166.
Malerba, M., & Cerana, R. (2016). Chitosan effects on plant systems. *International journal of molecular sciences*, *17*(7), 996.

Manabe, S., Wetherald, R. T., Milly, P. C., Delworth, T. L., & Stouffer, R. J. (2004). Century-scale change in water availability: CO 2-quadrupling experiment. *Climatic Change*, *64*(1), 59-76.

McDonald, M. B. (1999). Seed deterioration: physiology, repair and assessment. *Seed Science and technology*, *27*(1), 177-237.

Mejía-Teniente, L., Torres-Pacheco, I., González-Chavira, M. M., Ocampo-Velazquez, R. V., Herrera-Ruiz, G., Chapa-Oliver, A. M., & Guevara-González, R. G. (2010). Use of elicitors as an approach for sustainable agriculture. *African Journal of Biotechnology*, *9*(54), 9155-9162.

Miransari, M., & Smith, D. L. (2014). Plant hormones and seed germination. *Environmental and Experimental Botany*, *99*, 110-121.

Monaco, T. J., Weller, S. C., & Ashton, F. M. (2002). *Weed science: principles and practices*. John Wiley & Sons.

Mondal, M. M. A., Malek, M. A., Puteh, A. B., Ismail, M. R., Ashrafuzzaman, M., & Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. *Australian Journal of Crop Science*, *6*(5), 918.

Montesano, M., Brader, G., & Palva, E. T. (2003). Pathogen derived elicitors: searching for receptors in plants. *Molecular Plant Pathology*, *4*(1), 73-79.

Muñoz, G., Valencia, C., Valderruten, N., Ruiz-Durántez, E., & Zuluaga, F. (2015). Extraction of chitosan from Aspergillus niger mycelium and synthesis of hydrogels for controlled release of betahistine. *Reactive and Functional Polymers*, *91*, 1-10.

Muoni, T., Rusinamhodzi, L., & Thierfelder, C. (2013). Weed control in conservation agriculture systems of Zimbabwe: Identifying economical best strategies. *Crop Protection*, *53*, 23-28.

Muzzarelli, R. A. (1983). Chitin and its derivatives: new trends of applied research. *Carbohydrate Polymers*, *3*(1), 53-75.

Muzzarelli, R., Tarsi, R., Filippini, O., Giovanetti, E., Biagini, G., & Varaldo, P. E. (1990). Antimicrobial properties of N-carboxybutyl chitosan. *Antimicrobial agents and chemotherapy*, *34*(10), 2019-2023.

Nagar, R., Dadlani, P. M. and Sharma, S. P. (1998). Effect of hydro-priming on field emergence and crop growth of maize genotypes. Seed Sci Res. 26:1-5.

Nalewaja, J. D., Devilliers, B., & Matysiak, R. (1996). Surfactant and salt affect glyphosate retention and absorption. *Weed Research*, *36*(3), 241-247.

Nalewaja, J. D., Praczyk, T., & Matysiak, R. (1995). Surfactants and oil adjuvants with nicosulfuron. *Weed technology*, 689-695.

Narayanan, K. S., & Chaudhuri, R. K. (1992). Emulsifiable concentrate formulations for multiple active ingredients using N-alkylpyrrolidones. In *Pesticide Formulations and Application Systems: Eleventh Volume*. ASTM International.

Nge, K. L., Nwe, N., Chandrkrachang, S., & Stevens, W. F. (2006). Chitosan as a growth stimulator in orchid tissue culture. *Plant Science*, *170*(6), 1185-1190.

Nwe, N., Furuike, T., & Tamura, H. (2011). Production, properties and applications of fungal cell wall polysaccharides: chitosan and glucan. In *Chitosan for Biomaterials II* (pp. 187-207). Springer Berlin Heidelberg.

Omolehin, R. I., Ogunfiditimi, T. O., & Adeniji, O. B. (2007). Factors influencing adoption of chemical pest control in cowpea production among rural farmers in Makarfi Local Government Area of Kaduna State, Nigeria. *Journal of Agricultural Extension*, *10*.

Omura, Y., Shigemoto, M., Akiyama, T., Saimoto, H., Shigemasa, Y., Nakamura, I., & Tsuchido, T. (2003). Antimicrobial activity of chitosan with different degrees of acetylation and molecular weights. Biocontrol Science, 8(1), 25-30.

Ospina Álvarez, S. P., Ramírez Cadavid, D. A., Escobar Sierra, D. M., Ossa Orozco, C. P., Rojas Vahos, D. F., Zapata Ocampo, P., & Atehortúa, L. (2014). Comparison of extraction methods of chitin from Ganoderma lucidum mushroom obtained in submerged culture. BioMed research international, 2014.

Osswald, W., Niehuss, M., Huber, W., & Elstner, E. F. (1984). Support of non-host resistance by artificial leaf coating. Zeitschrift fuer Pflanzenkrankheiten und Pflanzenschutz, 91(4), 337-344.

Parera, C. A., & Cantliff, D. J. (1994). Presowing seed priming in" Horticultural Reviews volume 16" Edited by Jannick.

Pearson, B. A., Scott, R. C., & Carey III, V. F. (2008). Urea ammonium nitrate effects on bispyribac and penoxsulam efficacy. *Weed Technology*, *22*(4), 597-601.

Pichyangkura, R., & Chadchawan, S. (2015). Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae*, *196*, 49-65.

Pill, W. G., & Necker, A. D. (2001). The effects of seed treatments on germination and establishment of Kentucky bluegrass (Poa pratensis L.). Seed science and technology, 29(1), 65-72.

Placinta, C. M., D'mello, J. P. F., & Macdonald, A. M. C. (1999). A review of worldwide contamination of cereal grains and animal feed with Fusarium mycotoxins. *Animal feed science and technology*, *78*(1), 21-37.

Pospieszny, H. (1997). Antiviroid activity of chitosan. *Crop Protection*, *16*(2), 105-106.
Pospieszny, H., Chirkov, S., & Atabekov, J. (1991). Induction of antiviral resistance in plants by chitosan. *Plant Science*, *79*(1), 63-68.

Pospieszny, H., Struszczyk, H., & Cajza, M. (1996). Chitin Enzymology. *Chitin enzymology, Muzarelli RAA ed., Atec, Italy*, *2*, 385-389.

Prashanth, K. V. H., Dharmesh, S. M., Rao, K. S. J., & Tharanathan, R. N. (2007). Free radical-induced chitosan depolymerized products protect calf thymus DNA from oxidative damage. *Carbohydrate research*, *342*(2), 190-195.

Qian, R. Q., & Glanville, R. W. (2005). *U.S. Patent No. 6,896,809*. Washington, DC: U.S. Patent and Trademark Office.

Quintana-Obregón, E. A., Plascencia-Jatomea, M., Sánchez-Mariñez, R. I., Burgos-Hernandez, A., González-Aguilar, G. A., Lizardi-Mendoza, J., & Cortez-Rocha, M. O. (2011). Effects of middle-viscosity chitosan on Ramularia cercosporelloides. *Crop Protection*, *30*(1), 88-90.

Raafat, D., Von Bargen, K., Haas, A., & Sahl, H. G. (2008). Insights into the mode of action of chitosan as an antibacterial compound. *Applied and environmental microbiology*, *74*(12), 3764-3773.

Rabea, E. I., Badawy, M. E. T., Stevens, C. V., Smagghe, G., & Steurbaut, W. (2003). Chitosan as antimicrobial agent: applications and mode of action. *Biomacromolecules*, *4*(6), 1457-1465.

Ramakrishna A., & Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior*, *6*(11), 1720-1731.

Ramos-García, M., Ortega-Centeno, S., Hernández-Lauzardo, A. N., Alia-Tejacal, I., Bosquez-Molina, E., & Bautista-Baños, S. (2009). Response of gladiolus (Gladiolus spp) plants after exposure corms to chitosan and hot water treatments. *Scientia Horticulturae*, *121*(4), 480-484.

Ramos-Solano, B., Algar, E., Garcia-Villaraco, A., Garcia-Cristobal, J., Lucas Garcia, J. A., & Gutierrez-Mañero, F. J. (2010). Biotic elicitation of isoflavone metabolism with plant growth promoting rhizobacteria in early stages of development in Glycine max var. Osumi. *Journal of agricultural and food chemistry*, *58*(3), 1484-1492.

Ranil, R. H. G., Niran, H. L., Plazas, M., Fonseka, R. M., Fonseka, H. H., Vilanova, S., ... & Prohens, J. (2015). Improving seed germination of the eggplant rootstock Solanum

torvum by testing multiple factors using an orthogonal array design. Scientia Horticulturae, 193, 174-181.

Rea, G., Antonacci, A., Lambreva, M., Margonelli, A., Ambrosi, C., & Giardi, M. T. (2010). The NUTRA-SNACKS project: basic research and biotechnological programs on nutraceutics. In *Bio-Farms for Nutraceuticals* (pp. 1-16). Springer US.

Rebeiz, C. A., Montazer-Zouhoor, A., Mayasich, J. M., Tripathy, B. C., Wu, S. M., Rebeiz, C. C., & Friedmann, H. C. (1988). Photodynamic herbicides. Recent developments and molecular basis of selectivity. *Critical reviews in plant sciences*, *6*(4), 385-436.

Reddy, A. R., Chaitanya, K. V., & Vivekanandan, M. (2004). Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. *Journal of plant physiology*, *161*(11), 1189-1202.

Reddy, M. B., Angers, P., Castaigne, F., & Arul, J. (2000). Chitosan effects on blackmold rot and pathogenic factors produced by Alternaria alternata in postharvest tomatoes. *Journal of the American Society for Horticultural Science*, *125*(6), 742-747.

Reddy, M. V. B., Arul, J., Angers, P., & Couture, L. (1999). Chitosan treatment of wheat seeds induces resistance to Fusarium graminearum and improves seed quality. *Journal of Agricultural and Food chemistry*, *47*(3), 1208-1216.

Rehman, H. U., Nawaz, M. Q., Maqsood, S., Ahmad, Basra., Irfan, Afzal., Azra, Yasmeen and Hassan, F. U. (2014). Seed priming influence on early crop growth, phonological development and yield performance of linseed (Linum usitatissimum L.). J Integr Agric. 13: 990-96.

Ribeiro, L. N., Alcântara, A. C., Darder, M., Aranda, P., Araújo-Moreira, F. M., & Ruiz-Hitzky, E. (2014). Pectin-coated chitosan–LDH bionanocomposite beads as potential systems for colon-targeted drug delivery. *International journal of pharmaceutics*, *463*(1), 1-9.

Richards, M. D., Holloway, P. J., & Stock, D. (1998). Structure-spray retention enhancement relationships for some polymers and polymeric surfactants. In *Proc. Fifth Int. Symp. on Adjuvants for Agrochemicals* (Vol. 1, pp. 79-84).

Rinaudo, M. (2006). Chitin and chitosan: properties and applications. Progress in polymer science, 31(7), 603-632.

Roller, S., & Covill, N. (1999). The antifungal properties of chitosan in laboratory media and apple juice. International Journal of Food Microbiology, 47(1), 67-77.

Roy, N. K. and Srivastava, A. K. (1999). Effect of presoaking seed treatment on germination and amylase activity of wheat (Triticum aestivum L.) under salt stress conditions. Rachis 18:46-51.

Ruhul Amin, A. R., Kucuk, O., Khuri, F. R., & Shin, D. M. (2009). Perspectives for cancer prevention with natural compounds. *Journal of Clinical Oncology*, *27*(16), 2712-2725.

Sadeghian, S. Y., & Yavari, N. (2004). Effect of water-deficit stress on germination and early seedling growth in sugar beet. *Journal of Agronomy and Crop Science*, *190*(2), 138-144.

Sahai, A. S., & Manocha, M. S. (1993). Chitinases of fungi and plants: their involvement in morphogenesis and host—parasite interaction. *FEMS Microbiology Reviews*, *11*(4), 317-338.

Sajomsang, W., Tantayanon, S., Tangpasuthadol, V., Thatte, M., & Daly, W. H. (2008). Synthesis and characterization of N-aryl chitosan derivatives. *International journal of biological macromolecules*, *43*(2), 79-87.

Sathiyabama, M., Akila, G., & Einstein Charles, R. (2014). Chitosan-induced defence responses in tomato plants against early blight disease caused by Alternaria solani (Ellis and Martin) Sorauer. *Archives of Phytopathology and Plant Protection*, *47*(14), 1777-1787.

Saw, N. M. M. T., Riedel, H., Kütük, O., Ravichandran, K., & Smetanska, I. (2010). Effect of elicitors and precursors on the synthesis of anthocyanin in grape (Vitis vinifera) cell cultures. *Energy Rec. J*, *1*, 189-192.

Seiichi, T., Yoshiaki, M., Masayoshi, J., & Norio, N. (1994). Induction of drug specific antibody and the controlled release of drug by 6-O-carboxymethyl-chitin. *Journal of Controlled Release*, *28*(1-3), 235-241.

Sharathchandra, R. G., Raj, S. N., Shetty, N. P., Amruthesh, K. N., & Shetty, H. S. (2004). A chitosan formulation Elexa™ induces downy mildew disease resistance and growth promotion in pearl millet. Crop Protection, 23(10), 881-888.

Sharma, G. P., Verma, R. C., & Pathare, P. B. (2005). Thin-layer infrared radiation drying of onion slices. *Journal of Food Engineering*, *67*(3), 361-366.

Shaxson, F., & Barber, R. (2016). *Optimizing soil moisture for plant production: The significance of soil porosity*. Rome, Italy: UN-FAO.

Shehata, S. A., Fawzy, Z. F., & El-Ramady, H. R. (2012). Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Australian Journal of Basic and Applied Sciences*, *6*(4), 63-71.

Silva, S. S., Menezes, S. M. C., & Garcia, R. B. (2003). Synthesis and characterization of polyurethane-g-chitosan. *European Polymer Journal*, *39*(7), 1515-1519.

Singh, H., Jassal, R. K., Kang, J. S., Sandhu, S. S., Kang, H., & Grewal, K. (2015). Seed priming techniques in field crops-A review. *Agricultural Reviews*, *36*(4), 251-264.

Spanoghe, P., De Schampheleire, M., Van der Meeren, P., & Steurbaut, W. (2007). Influence of agricultural adjuvants on droplet spectra. *Pest Management Science*, *63*(1), 4-16.

Struszczyk, M. H. (2001). Chitin and chitosan. Part II. Applications of chitosan (English version). *POLIMERY-WARSAW-*, *47*(6), 396-403.

Sutherland, F., & Walters, D. R. (2001). In Vitro Effects of Film-Forming Polymers on the Growth and Morphology of Pyrenophora Avenae and Pyricularia Oryzae. *Journal of Phytopathology*, *149*(10), 621-624.

Sutherland, F., & Walters, D. R. (2002). Effect of film-forming polymers on infection of barley with the powdery mildew fungus, Blumeria graminis f. sp. hordei. *European Journal of Plant Pathology*, *108*(5), 385-389.

Suvannasara, R. & Boonlertnirun, S. (2013). Studies on appropriate chitosan type and optimum concentration on rice seed storability. ARPN J. Agric. Biol. Sci. 8 (3) 196.

Suvannasara, S. B. R., Promsomboon, P., & Boonlertnirun, K. (2011). Application of chitosan for reducing chemical fertilizer uses in waxy corn growing. *Thai Journal of Agricultural Science*, *44*(5), 22-28.

Szymańska, E., & Winnicka, K. (2015). Stability of chitosan—a challenge for pharmaceutical and biomedical applications. *Marine drugs*, *13*(4), 1819-1846.

Talwar, H. S., Chandra Sekhar, A., & Nageswara Rao, R. C. (2002). Genotypic variability in membrane thermostability in groundnut. *Indian journal of plant physiology*, *7*(2), 97-102.

Tang, H., Zhang, P., Kieft, T. L., Ryan, S. J., Baker, S. M., Wiesmann, W. P., & Rogelj, S. (2010). Antibacterial action of a novel functionalized chitosan-arginine against Gram-negative bacteria. *Acta Biomaterialia*, *6*(7), 2562-2571.

Tester, M., & Bacic, A. (2005). Abiotic stress tolerance in grasses. From model plants to crop plants. *Plant Physiology*, *137*(3), 791-793.

Thadathil, N., & Velappan, S. P. (2014). Recent developments in chitosanase research and its biotechnological applications: A review. *Food chemistry*, *150*, 392-399.

Tikhonov, V. E., Stepnova, E. A., Babak, V. G., Yamskov, I. A., Palma-Guerrero, J., Jansson, H. B., ... & Varlamov, V. P. (2006). Bactericidal and antifungal activities of a low molecular weight chitosan and its N-/2 (3)-(dodec-2-enyl) succinoyl/-derivatives. *Carbohydrate polymers*, *64*(1), 66-72.

Tripathy, B. C., & Oelmüller, R. (2012). Reactive oxygen species generation and signaling in plants. *Plant signaling & behavior*, *7*(12), 1621-1633.

Tsai, G. J., Zhang, S. L., & Shieh, P. L. (2004). Antimicrobial activity of a low-molecular-weight chitosan obtained from cellulase digestion of chitosan. *Journal of food protection*, *67*(2), 396-398.

Uchida, Y., Izume, M., & Ohtakara, A. (1989). Preparation of chitosan oligomers with purified chitosanase and its application, in Chitin and Chitosan: Sources, Chemistry, Biochemistry, Physical Properties and Applications, G. Skjak- ˚ Bræk et al., Ed., pp. 373–382, Elsevier, London, UK.

Van der Lubben, I. M., Verhoef, J. C., Borchard, G., & Junginger, H. E. (2001). Chitosan and its derivatives in mucosal drug and vaccine delivery. *European Journal of Pharmaceutical Sciences*, *14*(3), 201-207.

Vargas, R. N., Hutmacher, B., & Wright, S. D. (2005). UC IPM pest management guidelines: cotton. *Statewide IPM Program. Oakland: Division of Agriculture and Natural Resources, University of California Publication*, *3444*.

Velez, C., Figueira, E., Soares, A. M., & Freitas, R. (2016). Native and introduced clams biochemical responses to salinity and pH changes. Science of the Total Environment, 566, 260-268.

Walters, D., Walsh, D., Newton, A., & Lyon, G. (2005). Induced resistance for plant disease control: maximizing the efficacy of resistance elicitors. *Phytopathology*, *95*(12), 1368-1373.

Wang, J. C., & Kinsella, J. E. (1976). Functional properties of novel protein: Alfalfa leaf proteins. Journal of Food Science, 41(2), 286–292.

Widstrom, N. W. (1996). The aflatoxin problem with corn grain. *The aflatoxin problem with corn grain.*, 219-280.

Wirsenius, S., Azar, C., & Berndes, G. (2010). How much land is needed for global food production under scenarios of dietary changes and livestock productivity increases in 2030?. *Agricultural systems*, *103*(9), 621-638.

Wirth, W., Storp, S., & Jacobsen, W. (1991). Mechanisms controlling leaf retention of agricultural spray solutions. *Pest Management Science*, *33*(4), 411-420.

Wongroung, S., Seesuriyachun, P., & Ruangpradith, P. (2002). Effect of chitosan sprays as a crop protection on Pe-tsai (Brassica campestris spp. pekinesis). *Advances in Chitin Science*, *479*, 481.

Xia, W., Liu, P., Zhang, J., & Chen, J. (2011). Biological activities of chitosan and chitooligosaccharides. *Food Hydrocolloids*, *25*(2), 170-179.

Xie, W., Xu, P., & Liu, Q. (2001). Antioxidant activity of water-soluble chitosan derivatives. *Bioorganic & Medicinal Chemistry Letters*, *11*(13), 1699-1701.

Xiong, L., Schumaker, K. S., & Zhu, J. K. (2002). Cell signaling during cold, drought, and salt stress. *The plant cell*, *14*(suppl 1), S165-S183.

Yen, M.T., Yang, J.H, Mau, J.L. (2009). Physicochemical characterization of chitin and chitosan from crab shells. Carbohydrate Polymers. 75, 15-21.

Yin, H., & Du, Y. (2010). Mechanism and application of chitin/chitosan and their derivatives in plant protection. In *Chitin, Chitosan, Oligosaccharides and Their Derivatives: Biological Activities and Applications* (pp. 605-617). CRC Press.

Younes, I., & Rinaudo, M. (2015). Chitin and chitosan preparation from marine sources. Structure, properties and applications. *Marine drugs*, *13*(3), 1133-1174.

Zargar, V., Asghari, M., & Dashti, A. (2015). A review on chitin and chitosan polymers: structure, chemistry, solubility, derivatives, and applications. ChemBioEng Reviews, 2(3), 204-226.

Zhang, M. I., Tan, T., Yuan, H., & Rui, C. (2003). Insecticidal and fungicidal activities of chitosan and oligo-chitosan. Journal of bioactive and compatible polymers, 18(5), 391-400.

Zhao, Q., Qian, J., An, Q., Gao, C., Gui, Z., & Jin, H. (2009). Synthesis and characterization of soluble chitosan/sodium carboxymethyl cellulose polyelectrolyte

complexes and the pervaporation dehydration of their homogeneous membranes. *Journal of Membrane Science*, *333*(1), 68-78.

Zhou, Y. G., Yang, Y. D., Qi, Y. G., Zhang, Z. M., Wang, X. J., & Hu, X. J. (2002). Effects of chitosan on some physiological activity in germinating seed of peanut. *Journal of Peanut Science*, *31*(1), 22-25.

Zhu, H., Dexter, R. W., Fox, R. D., Reichard, D. L., Brazee, R. D., & Ozkan, H. E. (1997). Effects of polymer composition and viscosity on droplet size of recirculated spray solutions. *Journal of Agricultural Engineering Research*, *67*(1), 35-45.

Ziani, K., Ursúa, B., & Maté, J. I. (2010). Application of bioactive coatings based on chitosan for artichoke seed protection. *Crop Protection*, *29*(8), 853-859.

Zivanovic, S., Basurto, C. C., Chi, S., Davidson, P. M., & Weiss, J. (2004). Molecular weight of chitosan influences antimicrobial activity in oil-in-water emulsions. *Journal of food protection*, *67*(5), 952-959.