Seminar report

MULTIFUNCTIONAL ROLE OF CHITOSAN IN HORTICULTURAL CROPS

By

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(2018-12-005)

Submitted in partial fulfilment of the requirement for course

PSMA 591: Master's Seminar (0+1)

DEPARTMENT OF PLANTATION CROPS AND SPICES

COLLEGE OF HORTICULTURE

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DECLARATION

I, Ann Sneha Baby (2018-12-005) hereby declare that the seminar entitled 'Multifunctional role of chitosan in horticultural crops' has been completed by me independently after going through the reference cited at the end and I haven't copied from any of the fellow students or previous seminar reports.

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25/01/2020 2018-12-005

CERTIFICATE

This is to certify that the seminar report entitled 'Multifunctionl role of chitosan in horticultural crops' has been solely prepared by Ann Sneha Baby (2018-12-005) under my guidance and has not been copied from fellow students.

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This is to certify that the seminar report entitled "**Multifunctional role of chitosan in horticultural crops"** is a record of seminar presented by **Ann Sneha Baby (2018-12-005)** on 17th January 2020 and is submitted for the partial requirement of the course PSMA 591.

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MULTIFUNCTIONAL ROLE OF CHITOSAN IN HORTICULTURAL CROPS

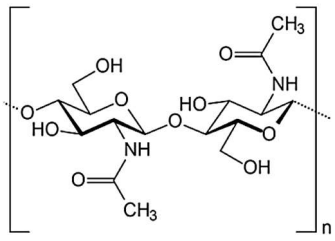
1. INTRODUCTION

In recent years, the always growing demand for food worldwide, the ongoing climate change, the dangerous consumption chemicals in farmlands and the increasing attention of consumers to high quality, safe and environmental friendly food products have stimulated the search for alternative biological methods that can meet this demand. Among the alternatives that are currently under investigation to avoid the use of chemical products to control plant diseases and increase crop productivity, are the biopolymer-based materials. In several cases, these materials have shown adequate activity against pathogens with low toxic effects on mammals and marginal impact on the environment. In addition, these biomaterials are also able to increase the productivity of many agricultural plants avoiding the use of large amounts of chemical fertilizers and dangerous farming practices. Among the tested biomaterials, the best results were obtained from those based on the biopolymer chitosan.

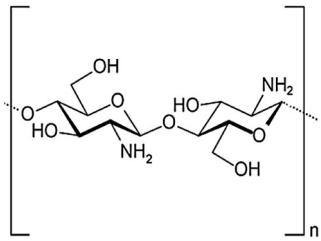
2. CHITOSAN

Chitosan is the deacetylated derivative of chitin. It is a natural polysaccharide, which can be produced after the alkaline deacetylation of chitin. Chitin is the second most abundant natural polymers, extracted from the exoskeleton of crustaceans, arthropods, as well as the cell walls of some fungi. Chitosan has been one of the most preferred biopolymers due to its biocompatibility, antioxidant, anticancer, biodegradability, antimicrobial and non-toxic properties as well as being an economical material, produced from waste resources such as seafood shells.

Plate 1: Chitin and chitosan



Chitin



Chitosan

3. SOURCES OF CHITOSAN

Commercial sources of chitosan are the shells of crustaceans such as shrimp, crab, lobster and krill. It is also obtained from molluscs such as squid pens, from fish scale, cell wall of fungi and exoskeleton of insects. The term chitosan does not describe a unique compound, but a group of commercially available copolymers. This heterogeneity can greatly affect the physical properties of chitosan, thus governing its biological applications.

Plate 2: Sources of chitosan

Shrimp

Crab

Lobster

Krill

Squid

Fish scales

Fungi

Insects

4. STRUCTURE OF CHITOSAN

Structurally, chitosan is a linear polymer, composed of two sub-units as D-glucosamine and N-acetyl-D-glucosamine, linked with each other through 1,4-glycosidic bonds. This biopolymer is obtained by partial deacetylation of chitin. The degree of deacetylation is generally defined as the glucosamine/ N-acetyl glucosamine ratio, which goes up as chitin is converted to chitosan. Therefore, when the percentage of N-acetyl glucosamine is higher than glucosamine, the biopolymer is called chitin and when the percentage of glucosamine exceeds N-acetyl glucosamine, the compound is called chitosan. Chitosan exhibits three functional groups, primary and secondary hydroxyl groups and amine groups. Due to these functional groups, they can easily undergo chemical modification. In addition, these functional groups affect the solubility and mechanical properties of chitosan. Chitosan also has 1,4-glycosidic linkages. Compared to chitin, chitosan is more soluble in acidic aqueous mediums. The solubility comes from the protonation of $–NH_2$ at the C-2 position of the D-glucosamine repeat

unit which induces the conversion of the polysaccharide to a polyelectrolyte in acidic media. The solubility character of chitosan broadens its scope of applications in almost every field of man's life and health such as agriculture, medical, process engineering, industries etc.

5. EXTRACTION OF CHITOSAN

Chitin from crustacean shell waste represents approximately half of the total weight of shell fish. During the processing, heads, shells and tails are considered inedible. The uncontrolled dumping of this waste biomass has a negative impact on the environment. Due to their abundance crustacean shells are considered as the source of choice for chitin isolation. From this chitin chitosan is obtained. Two types of methods are used to obtain chitosan, chemical and biological method.

5.1 Chemical method

After obtaining the shells from different sources, they are washed, dried and size reduced. The traditional chemical methods involve three steps: demineralization, deproteinisation and decolouration. The first step consists of processing the powdered raw material with an acidic treatment with HCl, the preferred reagent. The purpose is to remove mineral constituents such as calcium carbonate and calcium phosphate. An alkali treatment is used for deproteinisation of demineralized shells. Proteins are eliminated with NaOH. A decolouration step is added if a colourless product is wanted. Acetone or an organic solvent mixture are used to remove pigments such as carotenoids. After this step, chitin is obtained. Then it is mixed with 40-50% NaOH. Chitosan with different degree of deacetylation are generated depending on the reaction temperature, time and the concentration of alkali solution. The alkaline treatment hydrolyse the acetyl groups and transform the N-acetyl-D-glucosamine units into D-glucosamine units with free NH_2 groups.

Chemical method is the most commonly commercially used treatment due to its short processing time. But it have some drawbacks. The removed proteins and minerals, although potentially valuable supplements for human foods and animal feeds, are damaged that they are no longer appropriate for these applications. Also, it involve high cost because of the effluent treatment generated after acid and alkaline reagents. Therefore, the interest in biological extraction is increasing since it is safer and cheaper treatment. But to date, it has been limited to laboratory scale studies.

5.2 Biological method

To avoid acidic and alkali treatment that could be a source of environmental problems, biological treatments offer an alternative way to extract chitosan from crustacean shell. Lactic acid producing bacteria have been used for demineralization of crustacean shell. In fact, the lactic acid produced by bacteria reacts with calcium carbonate component in the biomass resulting in the formation of calcium lactate, which is precipitated and removed by washing. For the deproteinisation, proteases producing bacteria will eliminate proteins. The step of decolouration is same for biological method *ie.* by using acetone or other organic solvent mixtures. Chitin obtained is deacetylated to produce chitosan. Chitin acetyl groups are removed by chitin deacetylase enzyme. This is carried out by chitin deacetylase producing fungi or bacteria. This method has got several advantage over chemical method. It is environmentally safe and low cost because of the absence of effluents. Also, solubilized proteins and minerals which are obtained after the steps involved in the extraction procedure, can be used for human and animal nutrition.

6. APPLICATIONS OF CHITOSAN

Biopolymer chitosan has received much interest for potential wide application in agriculture due to its excellent biocompatibility, biodegradability and bioactivity. Apart from this it is also used in food industry, pharmaceuticals, biomedical applications, cosmetic and dermatological industry, textile industry and for waste water treatment.

7. MULTIFUNCTIONAL ROLE OF CHITOSAN IN AGRICULTURE

In agriculture, it has many functions such as enhancing growth and productivity (as a biostimulant), as an organic fertilizer and fertilizer protectant, as an elicitor of secondary metabolite production, against biotic and abiotic stresses and in post harvest management.

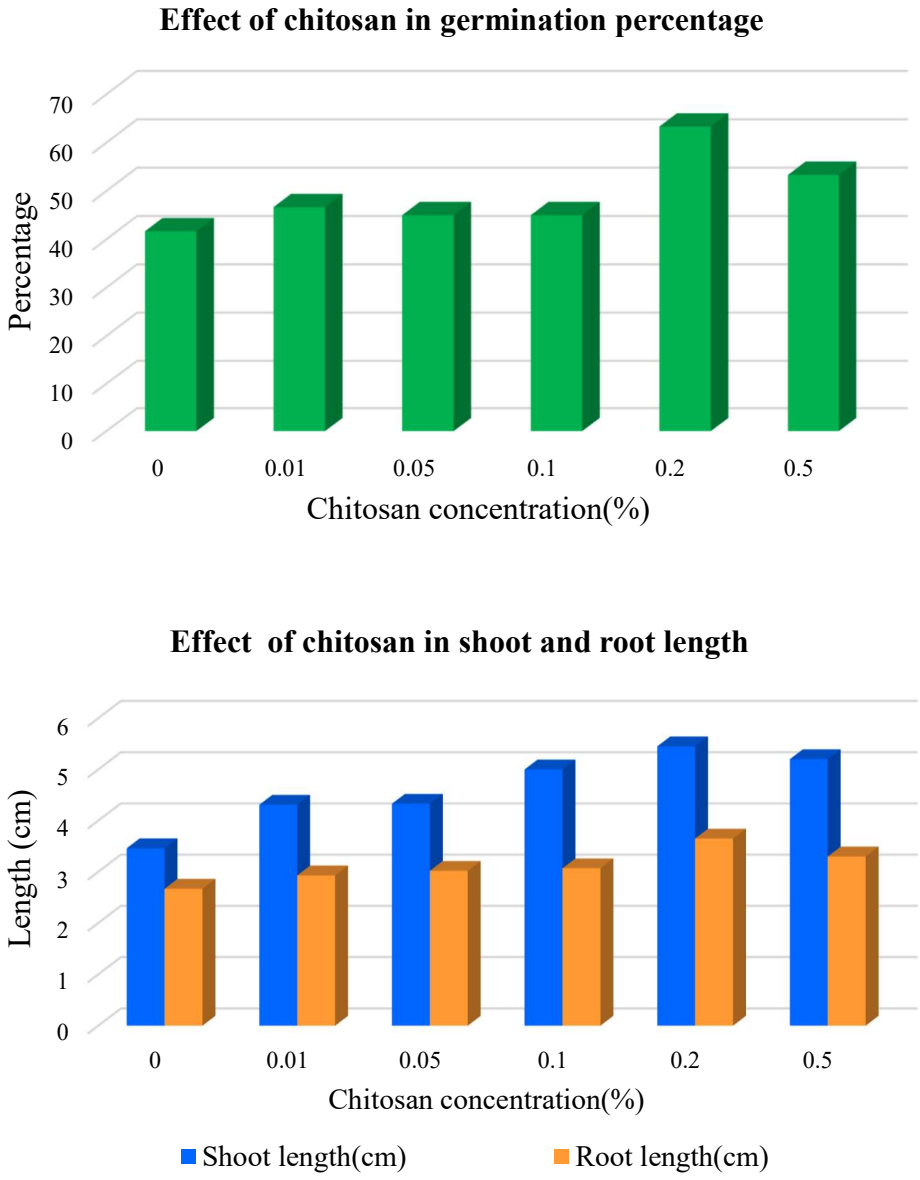
7.1 CHITOSAN AS A BIOSTIMULANT

Chitosan has been demonstrated to be a natural molecule that induces numerous biological responses in plants, dependent on its structure and concentration and on species and developmental stage of the plant. Chitosan use is recommended to enhance the

photosynthetic activity, vegetative growth, antioxidant activities, fruit quality attributes and overall growth and yield of the crop. Furthermore, it was reported to enhance plant growth and development in several plant systems. The mechanism for the biostimulant effect is not yet clear, but may involve, induced stimulation of antioxidant defence machinery, stimulation of nitrogen metabolism, increased uptake of water and essential nutrients through adjusting cell osmotic pressure and through improved water use efficiency by reduction of transpiration.

In order to study the effect of chitosan on germination and vegetative growth in Isabgol (*Plantago ovata* Forsk.), a pot experiment were conducted (Mahdavi, 2013). In the experiment the seeds were soaked in different concentrations of chitosan. The seeds of isabgol were soaked for 3 h in different solutions (0, 0.01%, 0.05%, 0.1%, 0.2% and 0.5% dissolved in one per cent acetic acid solution) of chitosan and then were air-dried. Twenty seeds were sowed in plastic pots that were filled with perlite and cocopit (1:1). After 10 days, the number of plants was reduced to four seedlings per pot. Germination percentages were recorded 10 days after seed sowing. Plants were harvested after 30 days of seed sowing and separated into shoot and root parts. Roots washed with tap water and blotted with paper towels before weighing. Dry weights of roots and shoots were determined after drying in the hot-air oven at 70° C for 24 h. The results demonstrated that the effects of chitosan concentrations were significant for germination percentage and length of shoot and root (Fig.1). Germination percentage increased with increment chitosan concentration. Plants pre-treated with 0.2 per cent chitosan showed highest germination percentage and growth in contrast to other plants. The increase of length of shoot and root was significant at 0.2 per cent and 0.5 per cent chitosan. Also, weight of shoot and root increased when the chitosan concentration was raised. The highest dry weight of shoot and root was observed at 0.2 per cent chitosan.

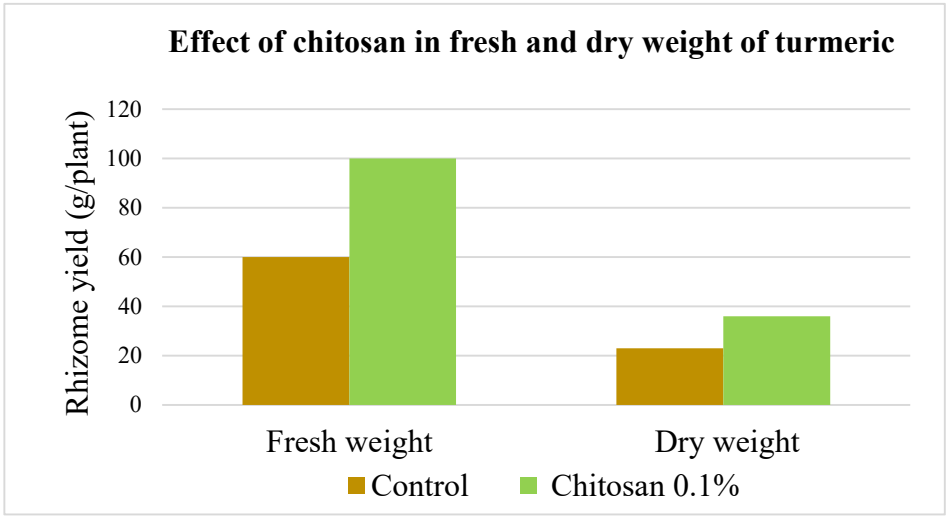
Fig. 1: Effect of chitosan seed treatment on germination percentage, shoot and root length in isabgol.

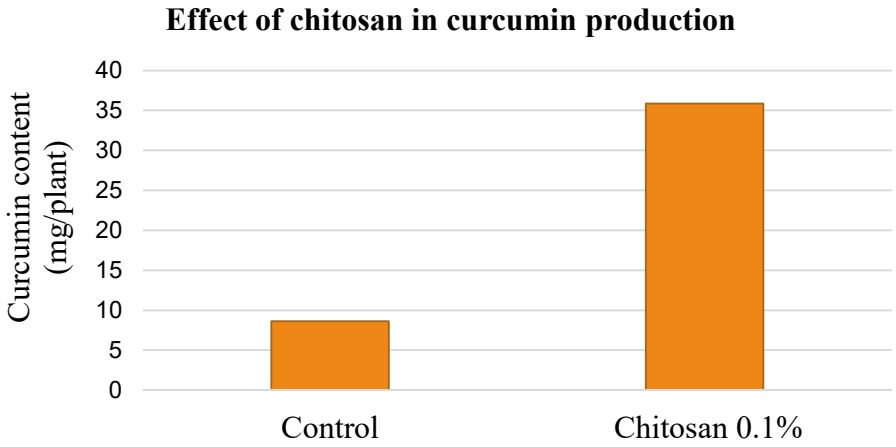


Field experiments were conducted to investigate the effect of foliar application of chitosan on growth, yield attributes and curcumin content of turmeric (Anusuya and Sathiyabama, 2016). Chitosan 0.1 per cent was sprayed at a regular interval of 30 days up to 210 days. Results revealed that the growth parameters were increased with the application of chitosan. This resulted in increased rhizome yields. Foliar application of chitosan increased the fresh weight of rhizome by 60% and dry weight by 50%. Chitosan treatment elicited 4 fold increase in curcumin content compared to control (Fig. 2). It is concluded that foliar

application of chitosan at a concentration of 0.1 per cent at vegetative stage enhanced the plant growth and development, which resulted in increased rhizome yield in turmeric. Hence, chitosan can be used as an eco-friendly compound to protect turmeric plants as well as to enhance yield and curcumin content under field condition.

Fig. 2: Effect of chitosan on yield and curcumin content in turmeric





Mondal *et al*., (2012) conducted pot and field experiments to investigate the effect of foliar application of chitosan, a growth promoter, on morphological, growth, yield attributes and fruit yield of okra. The experiment comprised of four levels of chitosan concentrations *viz*., 0 (control), 50, 100 and 125 ppm. The chitosan was sprayed three times at 25, 40 and 55 days after sowing. The pot experiment was laid out in a completely randomized design and the field experiment in a randomized complete block design, both with four replicates.

Results revealed that most of the morphological (plant height, leaf number plant^{-1}), growth (total dry mass plant^{-1}, absolute growth rate, relative growth rate), and yield attributes (number of fruits plant^{-1} and fruit size) were increased with increasing concentration of chitosan until 125 ppm, resulted the highest fruit yield in okra (27.9% yield increased over the control). However, the increment of plant parameters as well as fruit yield was not significant from 100 ppm of chitosan. Therefore, foliar application of chitosan at 100 or 125 ppm may be used at early growth stage to achieve a maximum fruit yield in okra. Results revealed that number of fruits plant^{-1}, fruit length and diameter and single fruit weight were higher in chitosan applied plants than control (Table 1). Results showed that yield attributes and fruit yield increased significantly with increasing concentration of chitosan up to 100 ppm. The higher fruit yields both per plant and per hectare were recorded in 100 and 125 ppm chitosan treatments, in which the highest values recorded in 125 ppm (15.31 t ha^{-1}). The fruit yield was higher in 100 and 125 ppm chitosan treatments due to production of higher number of fruits plant^{-1} and increased fruit size. Again, the number of fruits plant^{-1} increased in chitosan applied plants than control due to increase the plant height, resulting from increase in the fruit bearing nodes in okra. In contrast, the lowest fruit yield was recorded in control plants of both pot and field conditions due to production of fewer fruits and smaller fruit size.

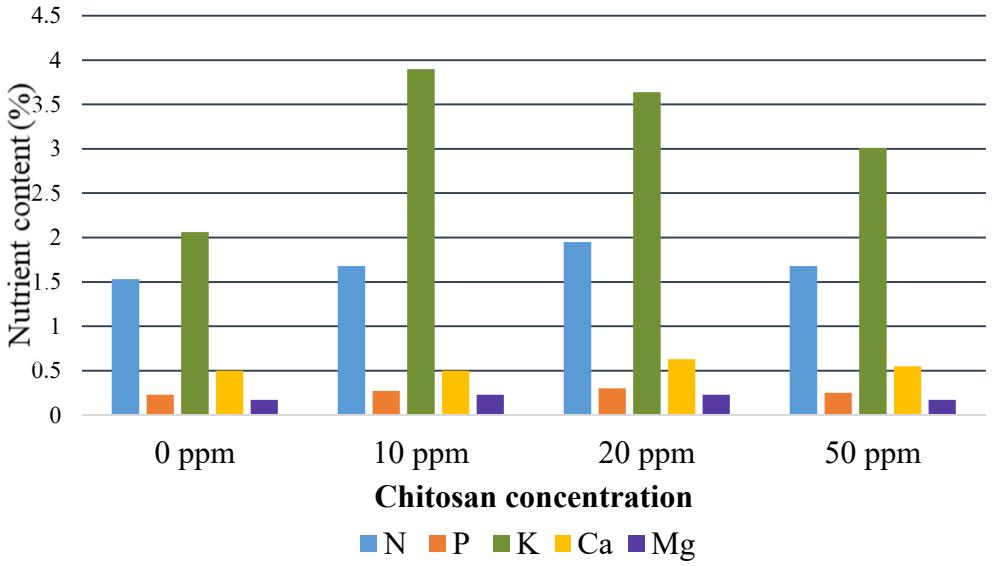
Table 1: Effect of foliar application of chitosan on yield of okra

Chitosan concentration (ppm)	**No. of fruits per plant**	**Single fruit weight (g)**	**Fruit weight per plant (g)**	**Fruit yield (t ha^{-1})**
0	17.9	17.0	243	11.97
50	19.4	18.4	286	13.03
100	22.7	18.6	338	15.21
125	23.1	18.5	342	15.31

Effects of chitosan nanoparticles on biophysical characteristics and growth of Robusta coffee in green house condition has been investigated by Van *et al.* (2013). Chitosan nanoparticles were prepared by nano spray drier. Effect of chitosan on biophysical characters such as photosynthetic rate, transpiration of leaves, contents of chlorophyll, carotenoid, nutrient uptake and growth parameters of coffee were investigated. The results showed that chitosan nanoparticles enhanced uptake of nutrients such as 9.8-27.4% N, 17.3-30.4% P and

30-45% K (Fig.3). Also, treated seedlings had content of chlorophyll a, b and carotenoid higher than the control. Chitosan nanoparticles also had significant impact on the growth of the coffee seedlings and growth parameters.

Fig. 3: Effect of chitosan nanoparticles on nutrient uptake in coffee seedlings



Ramos-Garcia *et al*., (2009) studied the effect of chitosan in gladiolus yield. Gladiolus corms were dipped in chitosan 1.5% for 120 mins. Results indicated that chitosan treatment accelerated the plant emergence, augmented the number of flowers and cormlets and prolonged their vase life. Corms dipped in chitosan had the earliest emergence compared with the control. The emergence was accelerated by approximately two days compared to the control. The number of flowers per spike was considerably large in plants were corms were treated with chitosan. The vase life of gladiolus flowers were extended by approximately three days in treated plants and the number of cormlets per corm was doubled (Table 2).

Table 2: Effect of chitosan in gladiolus

Treatment	**Corm emergence (days)**	**No. of flowers per spike**	**Vase life (days)**	**Number of cormlets**
Control	10.2	6.4	2.8	2.3
Chitosan 1.5%	8.8	11.0	5.5	4.4

7.2 CHITOSAN AS AN ORGANIC FERTILIZER AND FERTILIZER PROTECTANT

Chitosan can be considered as an organic fertilizer as it contain 6-9% N. Also, it stimulate the growth of beneficial microbes in soil, as it acts as a carbon source and accelerates the conversion organic matter into inorganic form and thus improving the nutrient uptake. It also act as a chelating agent and thereby increasing availability of nutrients like iron, copper and zinc. It also increases water holding capacity because of its highly porous naature and due to all these reasons an improvement in crop growth can be achieved by soil application of chitosan (Xu and Mou, 2018).

Chitosan when applied to soil as an organic fertilizer, enhanced the growth of chilli seedlings (Chookhongkha *et al*., 2012). Seedlings grown in the soil mixed with 1.0 per cent chitosan presented the greatest growth rate and chlorophyll content, and a higher number of dark green. A comparison of chitosan concentrations at 0.5 and 1.0 per cent (w/w) on the growth and seed productivity was also performed in the green house. The chili seedlings cultured in soil with 1.0 per cent chitosan powder presented significantly higher plant heights, canopy diameter, leaf numbers plant^{-1}, leaf widths and lengths, chlorophyll content and dark green leaf color as compared to the control plants. As a result, the significantly greatest seed yield indicated by fruit fresh weight plant^{-1}, fruit numbers plant^{-1}, seed numbers fruit^{-1}, and seed weight plant^{-1} was observed in the plants grown in the soil mixed with 1.0 per cent of chitosan. The seed yield components were significantly increased with a higher concentration. This suggests that the concentration plays an important role to the increase in fruit and seed yield. As a result, the chili growth and seed yield are greater in the soil mixed with chitosan (Table 3). The 1.0% of chitosan, however, is the most effective for the chili growth.

Table 3: Effect of soil application of chitosan in chilli growth

Treatments	Plant height (cm)	Canopy diameter (cm)	Leaf number per plant	Chlorophyll content
Control	18.58	10.20	114.40	26.82
Chitosan 0.5%	43.57	27.98	126.45	24.98
Chitosan 1.0%	46.27	32.23	156.25	40.75

Chitosan nanoparticles can be used to impart, control release property to fertilizers. At present, it is estimated that the intended target plants cannot absorb about 80%–90% of phosphorus, 40%–70% of nitrogen and 50%–70% of potassium contained in fertilizers. This resource loss is not only a waste of money but it also results in severe environmental pollution. Chitosan nanoparticles offers a solution for it. Wu and Liu (2007) prepared chitosan coated NPK compound fertilizer with controlled release and water retention property. Structure of this granule was the three layer, which the inner core is NPK compound fertilizer, middle coating is chitosan and outer coating is P(AA-co-AM) superabsorbent polymer. The middle layer chitosan, served as a physical barrier for mass transfer and reduced the rate of water diffusion into the core and the nutrient diffusion outside the core, thus providing the product with a good controlled release property.

7.3 CHITOSAN AS AN ELICITOR IN SECONDARY METABOLITE PRODUCTION

The elicitation effect of chitosan in aswagandha was studied (Gorelick *et al*., 2015). Aswagandha plants were grown under hydroponic conditions and elicitors such as NaCl and chitosan were provided through the hydroponic nutrient media. Chemical analysis of treated plants using HPLC revealed that elicitation for 24 h significantly increased the content of bioactive compound, withaferin A. Results have shown that chitosan has increased the withaferin A content by 69 per cent than the control (Fig. 4).

Lei *et al*. (2011) reported that, foliar application of chitosan 100 mg/L increased the artemisinin content in *Artemisia annua* L (Fig. 5). Following the treatment, the artemisinin content started to increase after 4 h and reached its maximum amount at 48 h. The content of dihydroartemisinic acid was significantly higher than that of control at 8 h after chitosan treatment, and increased by 72 per cent at 24 h. Foliar application of chitosan did not influence the growth of Artemisia over 16 days investigation period, also no significant differences in dry weight and height were observed.

There are so many reports based on the elicitation activity of chitosan. Chitosan 200 mg L^{-1} when added to suspension cultures of *Mentha piperita,* improved the menthol production by 40 fold (Chang *et al.,* 1998). Also, seed soaking and root dipping in 1 per cent chitosan before the transplanting of sweet basil resulted in increased growth and secondary

metabolite contents, along with a 2.5% and 2% increase in rosmarinic acid and eugenol levels respectively (Kim *et al.,* 2005). In oregano, chitosan at a concentration of 200 mgL^{-1} upregulated the polyphenol content (Yin *et al*., 2012).

Fig. 4: Elicitation effect of chitosan in aswagandha plants

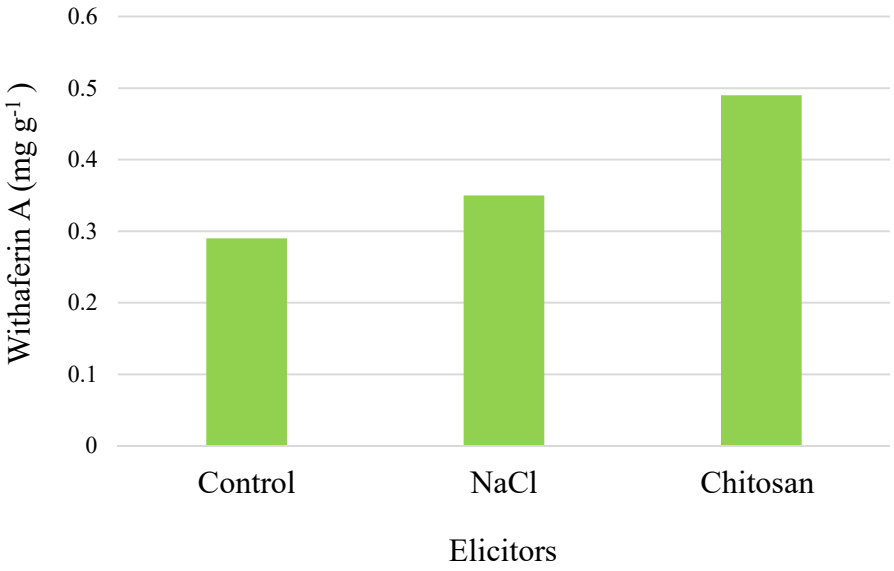
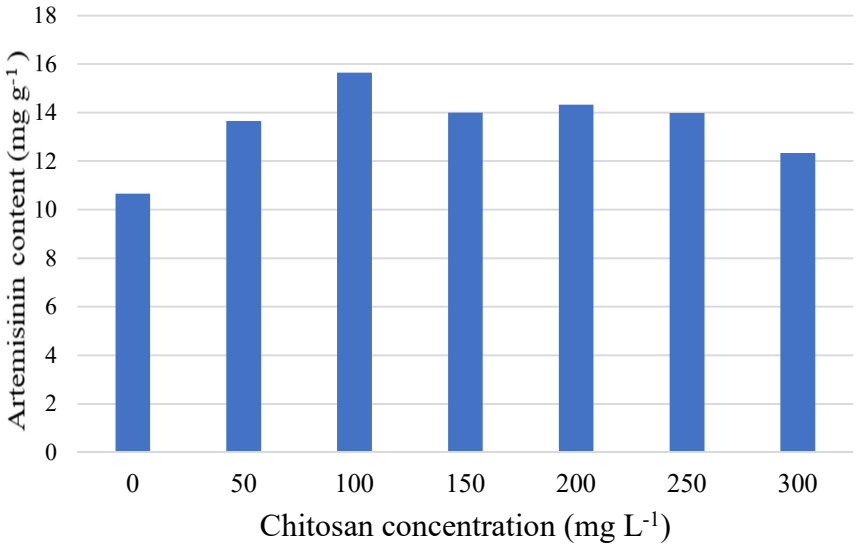


Fig. 5: Effect of chitosan in biosynthesis of artemisinin in *Artemisia annua*



7.4 CHITOSAN AGAINST BIOTIC AND ABIOTIC STRESSES

Recent studies have shown that chitosan induces mechanisms in plants against various biotic (fungi, bacteria, virus, insects and nematodes) and abiotic (salinity, drought, salinity and cold) stresses

7.4.1 Chitosan against plant pathogens

Chitosan act as a plant defense booster against biotic stresses by enhancing plant immunity and through anti-microbial activity.It act as an elicitor of plants defence responses through various mechanisms such accumulation of pathogenesis related proteins such as chitinase and beta-1,3-glucanase, by induction of plant defence enzymes such as phenyl alanine ammonia lyase, by scavenging of reactive oxygen species and through callose deposition and ligin biosynthesis. Anti microbial activity includes anti bacterial, anti fungal and anti viral mechanisms caused by chitosan. Three antibacterial mechanisms of chitosan were suggested such as ionic surface interaction resulting in cell wall leakage, binding of chitosan to the genetic material of microorganism and thereby inhibiting their protein and mRNA synthesis and the formation of an external film over the plant surface, limiting the nutrient availability for microorganisms (Goy *et al*., 2009). Ghaouth *et al*.(1992) suggested that anti-fungal mechanism of chitosan is by inhibiting spore germination, germ tube elongation and radial growth. Chirkov *et al*. (2001) reported anti-viral activity of chitosan. Chitosan treatment of plants may decrease inoculation efficiency, inhibit virus multiplication, or constrain virus transport within the plant. Chitosan-induced callose deposition and ribonuclease activity are related to the mechanism of antiviral resistance.

The effects of chitosan against the wilt pathogen of tomato, *Ralstonia solanacearum* were evaluated under greenhouse conditions (Algam *et al*., 2010). Chitosan applied as soil drench (SD) or seed treatment (ST), both at a concentration of 10 mg/L, significantly reduced wilt incidence by 72% and 48%, respectively. In addition, it was found that, the biocontrol efficacy of chitosan was increased when applied as a soil drench as compared with seed treatment (Fig. 6).

The ability of chitosan (CS) and oligochitosan (OCS) to enhance ginger (*Zingiber officinale*) resistance to rhizome rot caused by *Fusarium oxysporum* in storage was investigated (Liu *et al*., 2016). Both chitosan and oligochitosan at 1 and 5 g/L significantly

inhibited rhizome rot, with the best control at 5 g/L. Chitosan and oligochitosan treatments at 1 g/L significantly decreased the disease index of ginger rhizome relative to the control, with the chitosan treatment providing better control than the oligochitosan treatment. The control effect for both compounds increased when a concentration of 5 g/L was used, though no significant difference between the chitosan and oligochitosan treatments was observed (Fig. 7).

Fig. 6: Effect of chitosan against bacterial wilt in tomato

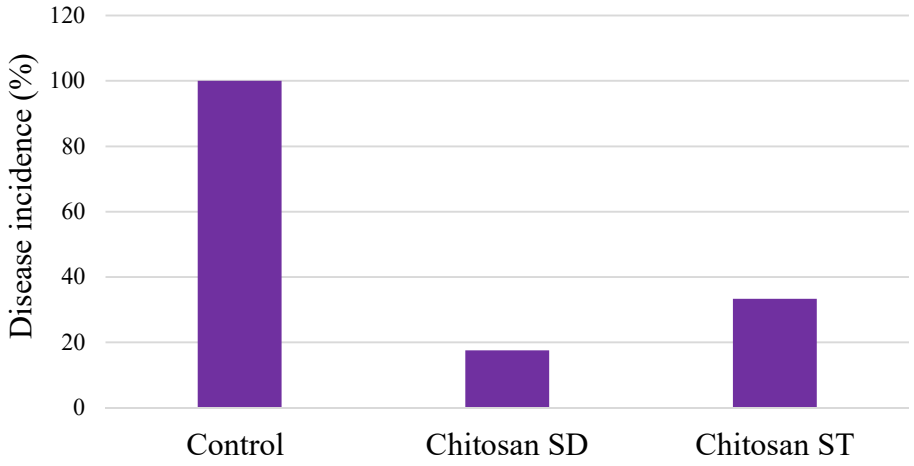
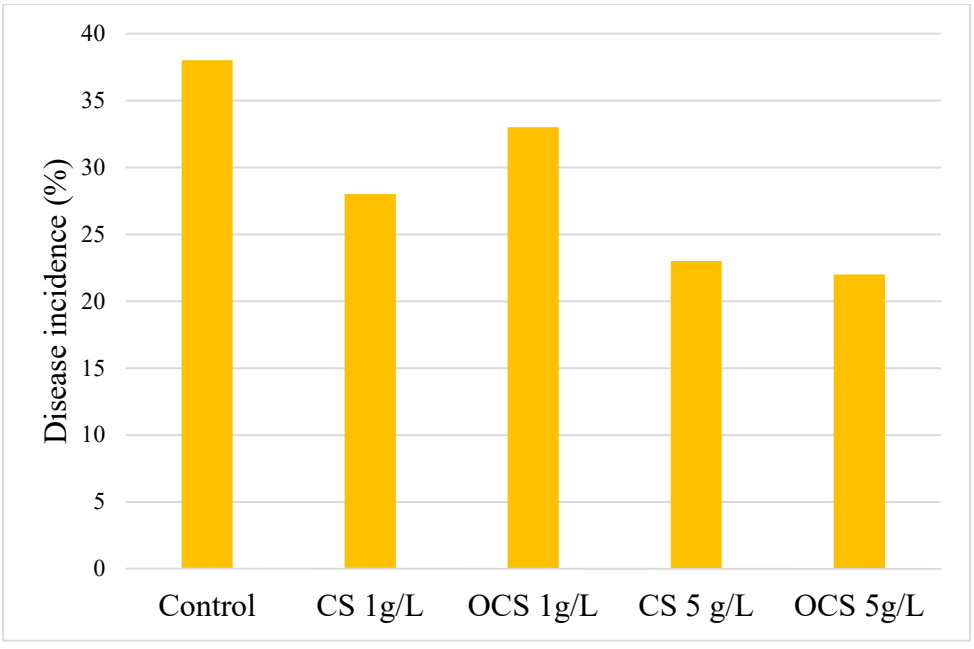


Fig. 7: Effect of chitosan and oligochitosan against rhizome rot of ginger in storage



7.4.2 Insecticidal activity of chitosan

Chitosan was found to be lethal against larvae of leaf worm, *Spodoptera littoralis* (Rabea *et al*., 2005). The insecticidal activity was tested against third-instar larvae of *S. littoralis* by feeding the larvae on artificial diet containing 5g/kg of test compound. This resulted in 100 per cent mortality. This high insecticidal activity was confirmed by exposing the larvae to cauliflower plants and spraying with chitosan derivative at a concentration of 5g/L. This resulted in 75 per cent mortality after 4 days. Chitosan 3g/L was effective in controlling *Helicoverpa armigera*. When sprayed on infected leaf, the mortality was 38.4 per cent after 24 h and 40 per cent after 72 h. Chitosan 3g/L resulted in 72 per cent mortality after 48h against *Plutella xylostella*. This is comparable to commercial pesticides (Zhang and Tan, 2003).

7.4.3 Nematicidal activity of chitosan

Asif *et al*., (2017) studied the nematicidal effect of chitosan. Chitosan 1.5g/kg of soil was added to the potting mixture and each pot was inoculated with 1500 newly hatched second stage juveniles of root knot nematode. Results showed that chitosan application suppressed the nematode infestation. There was a decrease in eggmases/ plant and nematode population in 250g soil sample. In addition, chitosan application augmented plant growth characters such as height of the plant and resulted in increased yield (Table 4).

Table 4: Effect of chitosan against root knot nematode in brinjal

Treatments	**Egg masses/ plant**	**Nematode population in 250g soil sample**	**Height of plant (cm)**	**Yield (g)**
Control	162	1600	42	200
Chitosan (1.5g Kg^{-1})	75	800	66	290

Chitosan against abiotic stresses

Chitosan can mitigate the drought stress by promoting the activities of antioxidant enzymes such as super oxide dismutase and catalase, increasing proline accumulation, reduction of water potential and reduction in transpiration. And in case of heat stress, an increase in ABA concentration and activation of heat shock resistant gene is associated with chitosan application. In case of salinity stress, a decrease in malondialdehyde and decrease in membrane leakage is seen with chitosan application.

7.4.4 Chitosan against drought stress

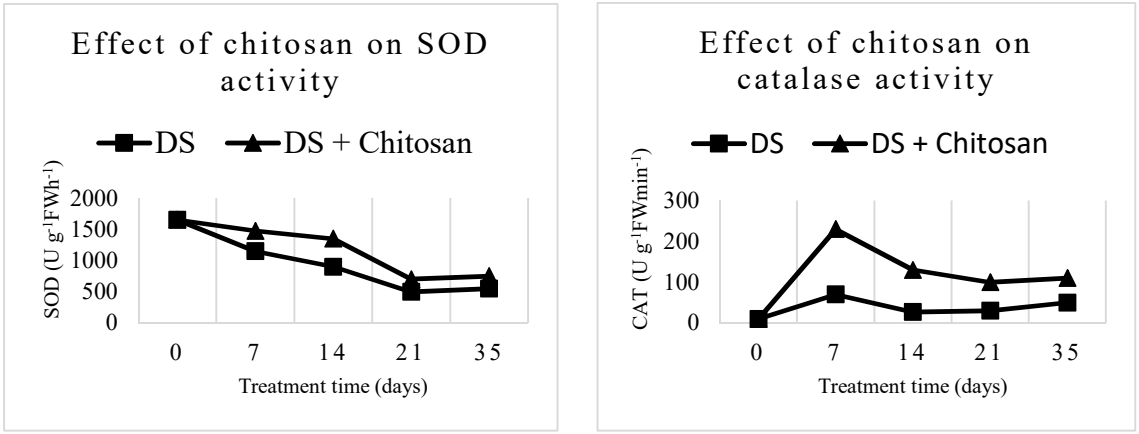
Drought stress or deficit irrigation limits the agricultural production causing many deleterious effects on plant health which mainly include the production of reactive oxygen species causing lipid peroxidation of membrane and interaction with other macromolecules, leading to reduced plant growth and yield. However, application of chitosan stimulated plant growth and increased the availability and uptake of water and essential nutrients, thereby contributing to enhanced reactive oxygen species (ROS) scavenging activities.

Under drought stress, proline accumulation increases. Proline is a vital osmoprotectant responsible for osmotic adjustment, quenching of ROS and maintenance of redox balance under abiotic stresses. Under severe drought stress, metabolic factor such as free proline content in leaves significantly increased as a part of an adaptive mechanism. Proline accumulation contributes to reduced water loss by lowering the leaf water potential. It also favors the water transport to leaves and increases their turgor. Treatment with chitosan has shown an increase in the accumulation of proline level. Membrane integrity is often disturbed when the plant is subjected to water deficit condition. Membrane relative permeability and MDA (malondialdehyde) concentration are used to represent membrane stability. In water deficit condition, there is an increase in MDA level which is a product of lipid peroxidation and may cause membrane leakage due to the accumulation of free radicals. However, chitosan functions as a positive regulator in osmotic adjustment and eliminate the adverse effect of drought stress symptoms. Due to the presence of abundant hydroxyl and amino groups present in chitosan, these react with ROS and form stable, non-toxic macromolecular radicals. Chitosan can scavenge OH and O^{2-} radicals and possesses DNA-protective properties. The ability to scavenge by chitosan may be attributed to its structure, which consists of hydroxyl and amino groups available to react with ROS. In response to drought, accumulation of total soluble sugar increases due to the breakdown of

polysaccharides which help in the maintenance of turgor. Sugar such as glucose and fructose contribute to drought resistance including signal transduction to modulate plant growth, development and stress responses. These might contribute to improved drought resistance *via* increase in osmotic adjustment and maintenance of carbon balance in response to dehydration stress. Also, drought stress impairs the photosynthetic ability, thus reducing chlorophyll synthesis. However, chitosan has been found to alleviate these effects, where chlorophylls and total carbohydrate increased when sprayed with chitosan. This may be due to increase in nitrogen and potassium content in plant shoot which helps in increasing the number of chloroplast per cell, thus contributing to increased synthesis of chlorophyll.

Yang *et al.* (2009) studied the effect of chitosan in alleviating drought stress in apple seedlings. It was suggested that, foliar application of chitosan 100mg/L is able to mitigate the effects of drought stress. Pre-treatment of apple seedling leaves with chitosan solution (20, 50, 100, 150 and 200 mgL^{-1}) prior to drought stress significantly decreased electrolyte leakage and the production of malondialdehyde in the leaves, while increasing antioxidant enzyme activities (superoxide dismutase and catalase), following imposition of drought stress conditions. An optimum response was obtained at chitosan concentration of 100 mgL^{-1}. When apple seedlings were pre-treated with 100 mg l^{-1} of chitosan, cell membrane stability and antioxidant enzyme activities were enhanced for 21 days of drought treatment (Fig. 8). Following restoration of moisture and a repeated drought stress, similar results were obtained on day 35. It is proposed that chitosan may act as an exogenous antioxidant that enhances resistance to oxidative stress during drought.

Fig. 8: Effect of chitosan on superoxide dismutase (SOD) and catalase activity (CAT)



7.4.5 Chitosan in alleviating heat stress

Heat stress is often considered a complicated issue as it usually coincides with drought stress and it's difficult to monitor these two stresses. However, there are reports that suggest ABA can trigger heat shock related genes like overexpression of ABF3 (Abscisic acid responsive-element-binding factor 3) could alleviate heat stress tolerance. Therefore, use of chitosan could overcome high-temperature stress by inducing ABA activity which is linked with the previous report on stomatal closure and further induces defence related ABA responsive genes.

Ibrahim and Ramadan (2015) conducted a study in *Phaseolus vulgaris* L. to study the effect of foliar sprays of zinc alone and zinc combined with chitosan and humic acid, on growth, nutrient elements content and yield in plants grown under heat stress. It has been reported that, chitosan treatment could be the best approach to escape heat stress in late- sown plants. The results of this study showed that foliar spray of zinc combined with chitosan alleviated the adverse effect of heat stress on yield performance and promoted the seed yield and harvest index (Table 5). This may be due to the important role of chitosan in increasing the permeability of plant membranes and enhancing the uptake of nutrients.

Table 5: Effect of chitosan in alleviating heat stress in dry bean

Treatments	N%	P%	K%	100 seed weight (g)	Harvest index
Control	2.4	0.18	2.30	47.20	49.50
Zn	2.95	0.29	2.86	49.80	51.10
Zn + Humic acid	3.53	0.39	3.25	51.50	52.20
Zn + Chitosan	3.74	0.41	3.32	52.70	53.90

7.4.6 Chitosan against salinity stress

Salinity affects whole plant, both physiologically and biochemically, and in severe cases, it also inhibits plant uptake of nutrients and water, either due to low external osmotic potential or toxic effect caused by higher accumulation of Na^+ and Cl^- ions as a result of direct ionic effects. Salt stress results in biochemical alteration and induces ROS which hampers the cellular machinery leading to oxidative stress. Various studies have found that

higher accumulation of MDA was observed in salt-affected plant which is primarily due to lipid peroxidation of membrane caused by ion toxicity. However, several researchers have reported that treatment with chitosan could alleviate the negative effects caused by salt stress.

In salt stress, plant exhibits a significant reduction in chlorophyll content due to the instability of protein complexes and accumulation of chlorophyllase (chlorophyll degrading enzyme). On the other hand, excessive accumulation of Na^+ caused by direct ionic effect induces stomatal closure which may reduce internal CO_2 and lead to the reduction of photosynthesis rate. However, upon seed treatment with oligochitosan, photosynthetic rate and stomatal conductance were found to increase since photosynthesis is dependent on the stomatal movement and protein associated with chlorophyll metabolism. In other study, foliar application of chitosan resulted in reduced stomatal conductance and photosynthetic rate with no change in internal CO_2 concentration. These contrasting results might be due to the fact that chitosan functionality is dependent on various factors such as method of application, degree of deacetylation, molecular weight and different species perceiving different responses. Production of ROS is obvious in plant subjected to stresses as discussed earlier. Plants have acquired their innate ROS scavenging mechanism by the production of enzymatic antioxidant compounds such as SOD, POD, and CAT and non-enzymatic antioxidant compounds. Higher accumulation of these enzymes indicates efficient detoxification of ROS.

Mahdavi (2013) conducted a study to find the effect of chitosan against salinity stress. Seeds of isabgol were subjected to four levels of salinity (0, 4, 8 and 12 dS/m) and two concentration of chitosan (0, 0.2%). Increase of salinity level decreased germination percentage, length and weight of shoot and root. When 0.2 per cent chitosan was given as seed treatment, it increased the germination percentage, root length and shoot length, than the control plants even at higher levels of salinity (Fig. 9,10). Therefore, chitosan ameliorated the adverse effects and partially improved plant tolerance to salinity stress conditions. The results showed that in unstressed plants, chitosan increased shoot length compared with control. Also, with increasing salinity to 12 dS/m, plants treated with chitosan were significantly taller than the control plants. The results showed that application of chitosan can reduce the harmful salinity and can promote germination and plant growth.

Fig. 9: Effect of chitosan in enhancing germination percentage in isabgol under salinity stress

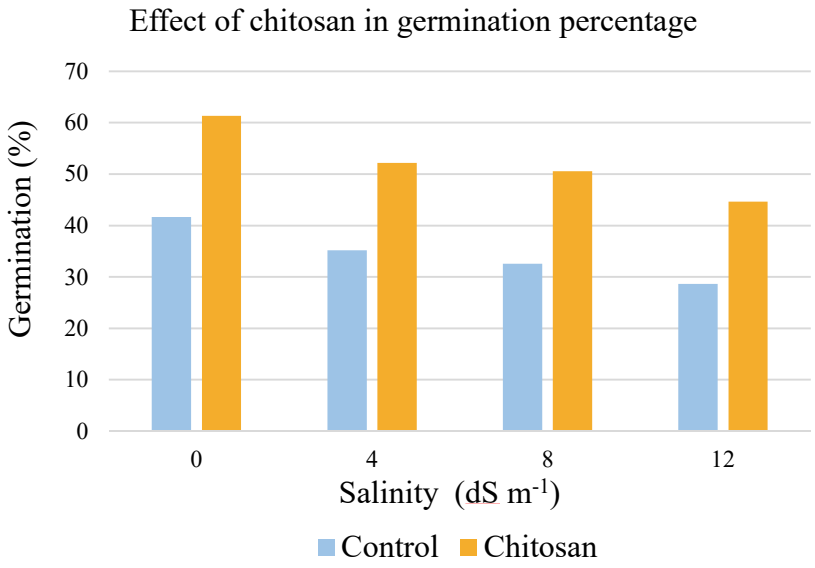
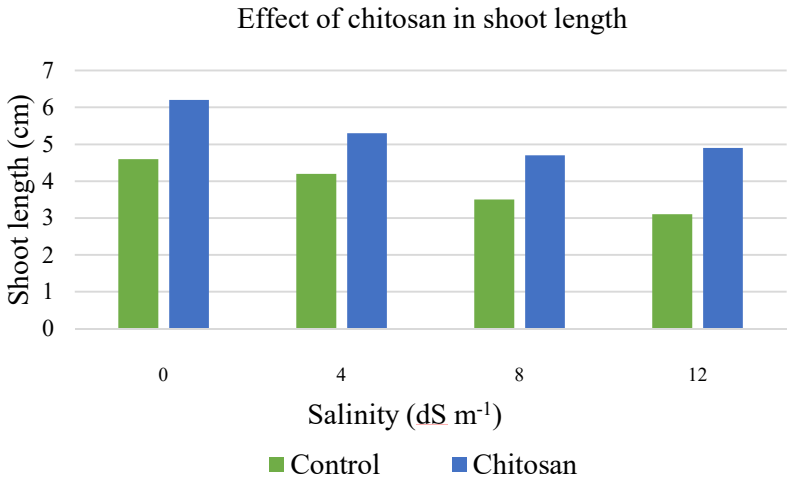
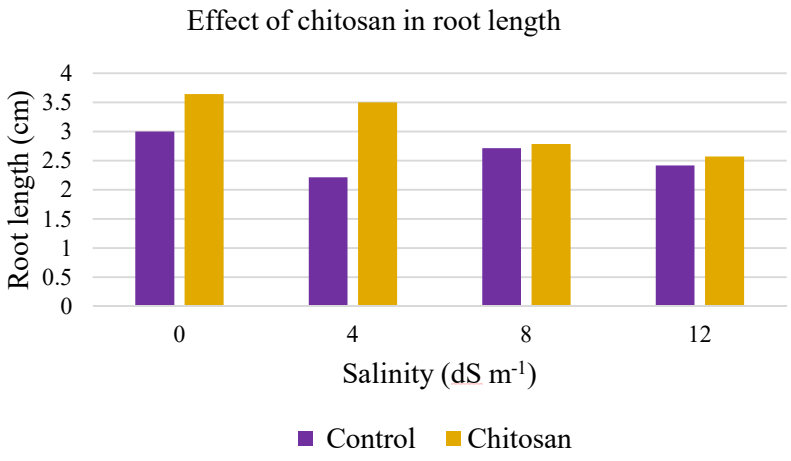


Fig. 10: Effect of chitosan on growth parameters of isabgol under salinity stress

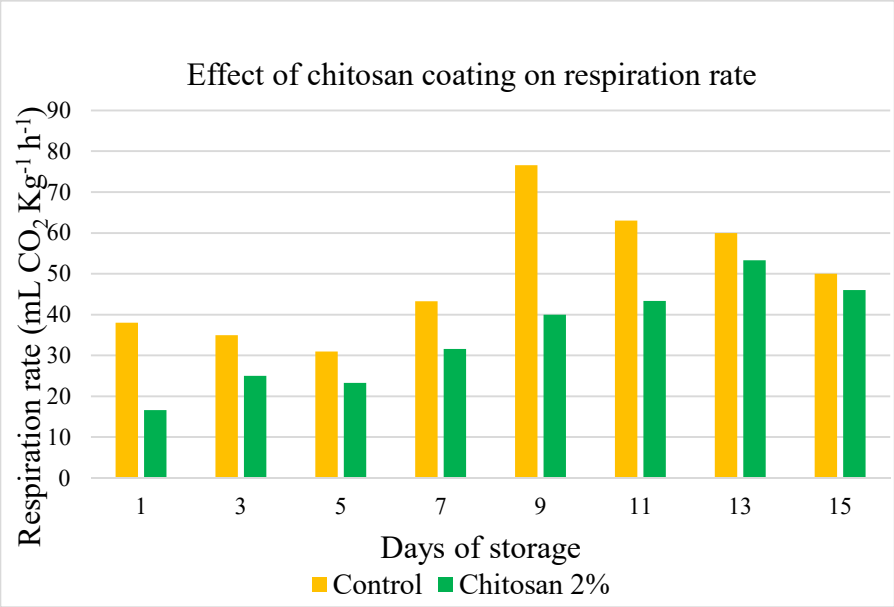




7.5 CHITOSAN IN POST HARVEST MANAGEMENT OF FRUITS AND VEGETABLES

Chitosan can be used in post harvest management as a coating, since it delay ripening and increase shelf life, keep the surface microbial growth under check, delay colour change, preserve water content, sustain fruit firmness, reduce weight loss percentage, improves antioxidant enzyme activity and preserve fruit quality. Zhu *et al*. (2018) reported 2% chitosan coating was effective in delaying ripening, extending postharvest life and reducing decay of mango fruits during storage. The decrease of firmness in the mango fruits was slowed down by chitosan coating. Firmness in the fruits treated with 2 per cent chitosan was 26.1 per cent higher than that of the control, even after 16 days of storage. Firmness in the control fruits rapidly decreased and was undetectable with the fruit firmness tester after 20 days of storage. Weight loss in the mango fruits was reduced by the coating with chitosan during storage. Weight loss in the fruits treated with 2.0% chitosan was 37.0% lower than that in the control after 15 days of storage. In addition, respiration rate in the mango fruits was inhibited by the chitosan treatment during storage (Fig. 11). The CO_2 production rate in the control fruits increased rapidly and reached its peak value on the ninth day after treatment, whereas the peak in the fruits coated with 2.0% chitosan was observed on the 13th day. The peak value in the fruits coated with 2.0% chitosan was 28.4 lower than that in the control.

Fig. 11: Effect of chitosan in inhibiting respiration rate in mango



8. CONCLUSION

The plethora of available and ongoing research on chitosan utilization, continues to present its efficacy. Chitosan, as a unique abundant biopolymer, has a promising future in development of sustainable agricultural practises as well as food production and preservation. However, more information about these processes are needed and thus this topic warrants further study.

9. DISCUSSION

1. Is chitosan water soluble?

No, chitosan is soluble only in dilute acids such as acetic acid.

2. Apart from agricultural uses, what all are the uses of chitosan. Elaborate?

Chitosan is used in pharmaceuticals as a carrier, due to its bio compatibility and non-toxicity. In food industry, it act as a functional food. Chitosan is having anti-oxidant property. It is also used to reduce obesity and cholesterol, correct anaemia, improve physical strength and appetite, *etc.* In biomedical industry, it is used in tissue regeneration. It also have anti-microbial property, so it can be used for wound dressing. In cosmetics, it is used in hair, skin and oral care products. It is also used for waste water treatment, as it an effective absorbent material for the removal of pollutants.

3. What is oligochitosan?

Oligochitosan is a derivative of chitosan. It is water soluble. It is obtained by hydrolysis of glycosidic bonds.

4. Which is better method for extraction of chitosan. Chemical or biological?

Now-a-days, chemical method is used in industries to produce chitosan. But it has some limitations. It is of high cost, due to the requirement of effluent treatment after the alkali and acid treatment. Also, the by products obtained such as proteins and minerals cannot be used again, as it get damaged due to the chemical methods adopted. Whereas, biological method is environment friendly. It costs low, since no effluents are generated. Also, the proteins and minerals obtained as a by product can be used in health supplements for humans and animals.

5. Any company producing chitosan in Kerala? How much does it cost?

Chitin and chitosan plant, under Matsyafed, situated in Kollam and Marine Hydrocolloids, Kochi, is producing and selling chitosan. Chitin and chitosan plant is providing it for Rs. 1000/kg**.**

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11. ABSTRACT

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Department of Plantation Crops and Spices
PSMA 591: Master's Seminar

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Admission No : 2018-12-005
Major Advisor : Dr. Jalaja S. Menon

Venue: Seminar hall
Date : 17-01-2020
Time : 11.30 am

Multifunctional role of chitosan in horticultural crops

Abstract

Chitosan is a cationic polysaccharide produced by the alkaline deacetylation of chitin. It is a linear polymer composed of two subunits, D-glucosamine and N-acetyl-D-glucosamine, linked with 1,4-glycosidic bonds. Commercial sources of chitosan are shells of crustaceans like shrimp, crab, lobster and krill. It can also be produced from squid pens, fish scales, fungi and insects. The extraction procedure involves demineralisation, deproteinisation, decolouration and deacetylation. Chitosan is a versatile biopolymer having several applications in agriculture, food, cosmetics, pharmaceuticals and biomedical industries.

In agriculture, chitosan is mainly exploited for enhancing crop production due to its bioactivities such as increasing seed germination, plant growth, chlorophyll content, photosynthetic rate and nutrient uptake and reducing stress and disease severity. Mahdavi (2013) reported that seed treatment in isabgol with 0.2 per cent chitosan increased germination percentage, shoot length and root length. Foliar spray of chitosan 0.1 per cent in turmeric increased the rhizome yield and curcumin content (Anusuya and Sathiyabama, 2016).

Chitosan can be used as an organic fertilizer. It acts as a carbon source for beneficial microbes in soil and accelerates the conversion of organic matter into inorganic forms, thus promoting the plant growth. In addition to this, chitosan nanoparticles when used as a coating over inorganic fertilizers, serves as a protectant and ensures slow release property. Chitosan is also reported to act as an elicitor in secondary metabolite production. Foliar application of chitosan 100 mgL^{-1} improved the artemisinin biosynthesis in *Artemisia annua* (Lei *et al*., 2011).

Chitosan induces mechanisms in plants against biotic and abiotic stresses and improves plant productivity. Chitosan and oligochitosan 5 gL^{-1}, when applied as dip, were effective against rhizome rot of ginger during storage (Liu *et al*., 2016). Ibrahim and Ramadan (2015) found that foliar spray of chitosan in combination with zinc on dry bean alleviated the harmful effects of heat stress and improved the seed yield and harvest index.

Chitosan when used as a coating on fruits and vegetables, delays ripening and colour change, reduces weight loss percentage, sustains fruit firmness, increases shelf life and preserves fruit quality. It is also effective in keeping surface microbial growth under check. Zhu *et al*. (2008) reported that two per cent chitosan coating was effective in delaying ripening and extending post-harvest life of mango fruits during storage.

Chitosan can be a good alternative to chemicals and pesticides, for improving crop productivity and imparting resistance to biotic and abiotic stress due to its diverse properties. It is a unique and abundant biopolymer, which has a promising future in the development of sustainable agricultural practices as well as food production and preservation.

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