Performance of Gamma Irradiated Polysaccharides as Growth Promoter on Selective Crops

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Dedicated to

My Parents

Declaration

I hereby declare that the work embodying this progress report entitled, **"Performance of Gamma Irradiated Polysaccharides as Growth Promoter on Selective Crops"** is carried out under the direct supervision of **Professor Dr. Ahmad Ismail Mustafa, Dr. Mubarak Ahmad Khan and Associate Professor Dr. Papia Haque.** I declare that this thesis, which I submit to Dhaka University for the award of **Doctor of Philosophy**, is my own personal effort. On the basis of this work, I have not already obtained a degree in Dhaka University or elsewhere. Furthermore, I took reasonable care to ensure that the work is original, and, to the best of my knowledge, does not breach copyright law, and has not been taken from other sources except where such work has been cited and acknowledged within the text. I declare that the work contained herein is my own except where explicitly stated otherwise in the text. Any contributions from others in the collaboration, such as diagrams or calibrations, are explicitly referenced in the text. Parts of this work have been published in **IJNME**.

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Performance of Gamma Irradiated Polysaccharides as Growth Promoter on Selective Crops

Abstract

Biopolymers are excellent to be used in various biomedical, environmental, industrial, pharmaceutical and agricultural applications. Chitosan and alginate are very forefront polymers of this category. Their potentiality as biofertilizer has been investigated in this study. Low molecular weights of these polymers were obtained after irradiating them at different radiation dose from 5-100 kGy by using Co^{60} gamma source. Optimized radiation dose for SA and CS were being used and a range of concentration from 100-1000 ppm of each of them and their mixture were applied by foliar spray on some crops (tomato, cabbage, cauliflower, spinach, mungdal, pumpkin, jute, eggplant, green chilli , red chilli and betel vine) over a period of time. For every cases, treated samples have shown more productivity and growth due to the fertilizer effect of the polymers compared to that of control samples. The treated plants other than betel vines have shown productivity and growth with a range of 16%-300% and 28%-300% respectively. However, betel vine has shown growth from 48%-81%. Lower concentrations of the polymer were found to be more effective than the higher concentration of them applied on the plants. TGA and SEM analysis confirmed that there was no stagnance of the polymers' over the plants. Analysis of nutrient and heavy metal uptake was also conducted for betel vines and for the vine's garden soil. Chitosan showed a better chelation effect with the metal cations, however, alginate was found to be superior in growth of the plants. Along with the increase of nutrients uptake into the plants, chitosan incorporated antifungal property in betel vines and from the sensory evaluation test it has been proved testier than the control plants.

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Abstract

Biopolymers are excellent to be used in various biomedical, environmental, industrial, pharmaceutical and agricultural applications. Chitosan and alginate are very forefront polymers of this category. Their potentiality as biofertilizer has been investigated in this study. Low molecular weights of these polymers were obtained after irradiating them at different radiation dose from 5-100 kGy by using Co^{60} gamma source. Optimized radiation dose for SA and CS were being used and a range of concentration from 100-1000 ppm of each of them and their mixture were applied by foliar spray on some crops (tomato, cabbage, cauliflower, spinach, mungdal, pumpkin, jute, eggplant, green chilli , red chilli and betel vine) over a period of time. For every cases, treated samples have shown more productivity and growth due to the fertilizer effect of the polymers compared to that of control samples. The treated plants other than betel vines have shown productivity and growth with a range of 16%-300% and 28%-300% respectively. However, betel vine has shown growth from 48%-81%. Lower concentrations of the polymer were found to be more effective than the higher concentration of them applied on the plants. TGA and SEM analysis confirmed that there was no stagnance of the polymers' over the plants. Analysis of nutrient and heavy metal uptake was also conducted for betel vines and for the vine's garden soil. Chitosan showed a better chelation effect with the metal cations, however, alginate was found to be superior in growth of the plants. Along with the increase of nutrients uptake into the plants, chitosan incorporated antifungal property in betel vines and from the sensory evaluation test it has been proved testier than the control plants.

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Chapter 1: Introduction

1. Introduction

Biopolymers are polymers produced by living organisms. Cellulose and starch, proteins and peptides, and DNA and RNA are all examples of biopolymers, in which the monomeric units, respectively, are sugars, amino acids, and nucleotides [1]. A biomaterial is any matter, surface, or construct, natural or man-made, which comprises whole or part of a living structure or biomedical device which performs, augments, or replaces a natural function that interacts with biological systems. Biomaterials can generally be produced either in nature or synthesized in the laboratory using a variety of chemical approaches utilizing metallic components or ceramics [2]. Different living organisms like shrimps, crabs, brown algae etc. produce some biomaterials in their body to tolerate adverse environmental conditions which have the plants growth promoting and anti-microbial capacity. Cellulose [3] is the most common biopolymer and the most common organic compound on Earth. About 33% of all plant matter is cellulose .The advantages of using these biomaterials [4] are that they are naturally available, cheap and have no destructive effect on overall environment including plants and animals which may be occurred in case of application of chemical fertilizers and pesticides. Chitosan (CS) [5] and sodium alginate (SA) are also two naturally occurring biomaterials extracted from crustacean shells and sea weeds respectively [6, 7].

Proper disposal of seafood wastes is a continuous problem throughout the world. Shrimp, crabs and other seafood processing plants continuously dumped their residues into landfills, creating management and environmental concerns associated with ground and drinking water pollution. Additionally, build up of seafood waste generates an unpleasant odor[8] and becomes an eye-sore to both tourists and local communities. On the other hand, brown algae are a wide source of natural biomaterials which have potential uses in agriculture and other sectors.

CS is a naturally-occurring linear polysaccharide derived from chitin, a major component of the shell of crustaceans and the second most abundant biopolymer in nature next to cellulose and is commercially available [7]. It has the potential in agriculture with regard to controlling plant diseases and promoting the plant growth. These molecules were shown to display toxicity and inhibit fungal growth and development. They were also reported to be active against viruses, bacteria and other pests. SA is a gum, extracted from the cell walls of brown algae. The chemical compound SA is the sodium salt of alginic acid (AA). Its empirical formula is $NaC_6H_7O_6$. It absorbs water quickly; it is capable of absorbing 200-300 times its own weight in water [6].

Irradiation can modify the viscosity, molecular weight, hydrophilic and mechanical properties of CS and SA resulting in enhanced properties. Previous work focused on the field test of CS as growth promoters for rice, red chili, potato, and carrot plants [9-11]. This treatment also increases the productivity of soybean (using Mitani and

Rajabasa varieties) in about 40% than control. Growth-promotion effect of radiation degraded SA on tea has also been studied in Vietnam, which indicates that a 100 ppm radiated SA causes an increase in the bud weight almost 35% [12]. Besides, it was also reported that SA possesses anti-fungal and disease defensive effect for plants. This research work is concerning on the effect of radiation processed biomaterials (CS and SA) on selective crops at different radiation doses and concentrations and make a study on treated and untreated plants.

1.1 Saccharides

Saccharide is another name of carbohydrate. The term carbohydrate derives from the fact that many of them have a formula that can be simplified to $(CH_2O)n$. On the basis of the number of forming units, three major classes of carbohydrates can be defined: monosaccharides, oligosaccharides and polysaccharides.

- **Monosaccharides** or simply sugars are formed by only one polyhydroxy aldehydeidic or ketonic unit. The most abundant monosaccharide is glucose, also called dextrose.
- **Oligosaccharides** are formed by short chains of monosaccharidic units (from 2 to 20) linked one to the next by chemical bonds, called glycosidic bonds. The most abundant oligosaccharides are disaccharides, formed by two monosaccharides, and especially in the human diet the most important are sucrose (common table sugar), lactose and maltose. Within cells many oligosaccharides formed by three or more units do not find themselves as free molecules but linked to other ones, lipids or proteins, to form glycoconjugates.
- **Polysaccharides** are polymers consisting of 20 to 10^7 monosaccharidic units; they differ each other for the monosaccharides recurring in the structure, for the length and the degree of branching of chains or for the type of links between units. In the plant kingdom several types of polysaccharides are present, in vertebrates there are only a small number.The most common polysaccharides consisting of single monosaccharides are starch, glycogen, celluose, chitin and alginate.

1.2 Comparison between polysaccharides with cellulose

Cellulose is the major polysaccharide found in plants responsible for structural role. Chitin, CS and SA are polysaccharides and also natural polymers “biopolymers”.

Chitin is one of the most abundant linear homopolysaccharides (long chain polymer), having repeated units of N-acetyl-D-glucosamine and is insoluble in water. These subunits form beta-glycosidic linkages similar to those formed by glucose molecules in cellulose. In fact, the only chemical difference from cellulose is the replacement of a hydroxyl group at C-2 with an acetylated amino group. Chitin can thus be described as cellulose, but simply with a different group at the second carbon. This increases hydrogen bonding, resulting in stronger molecules. Chitin is the exoskeleton of many

arthropods, and is the main component of cell walls in fungi, radulas of mollusks etc. Like cellulose, it is indigestible by vertebrate animals. Chitin has also been used as surgical thread, making it very valuable. CS is a polymer with random units of D-glucosamine and N-acetylglucosamine, and alginate is a polymer with repeating unit of β-D-manuronil linked with α-L-guluronil and basically the deactylated product of chitin. In order to make it soluble, deacetylation is carried out using. Their natural biological compatibility & activity rendered them as promising candidates for various biomedical applications. CS is derived from chitin, a type of polysaccharide that is found in the hard exoskeletons of shellfish like shrimp & crab. Chitin is one of the most abundant polysaccharides found in nature, making CS a plentiful & relatively inexpensive product.

SA is the purified carbohydrate product extracted from brown seaweeds by the use of dilute alkali. It consists chiefly of the sodium salt of AA, a polyuronic acid composed of b-D-mannuronic acid residues linked so that the carboxyl group of each unit is free while the aldehyde group is shielded by a glycosidic linkage. It contains not less than 90.8 percent and not more than 106.0 percent of SA of average equivalent weight 222.00. Like CS, SA can be processed easily in water and is quite non-toxic & non-inflammatory; that is why it has been utilized in some countries for wound dressing & for use in food products.

Cellulose is one of the most naturally abundant organic compounds found on the planet. Cellulose is an unbranched polymer of glucose residues put together via β-1,4-glycosidic bonds, which allow the molecule to form long and straight chains. This straight chain conformation is ideal for the formation of strong fibers. Cellulose is insoluble in water and aqueous solutions. It forms crystals and hydrogen bonds with amino acids. This quality of using intra and intermolecular hydrogen bonds to make crystals renders cellulose excessively insoluble in water and aqueous solutions. However, individual strands of cellulose aren't very hydrophobic as compared to other polysaccharides. It is the property of forming crystals that makes cellulose so insoluble. Figure 1 shows the comparison between the structures [13] of chitin, CS, SA with Cellulose.

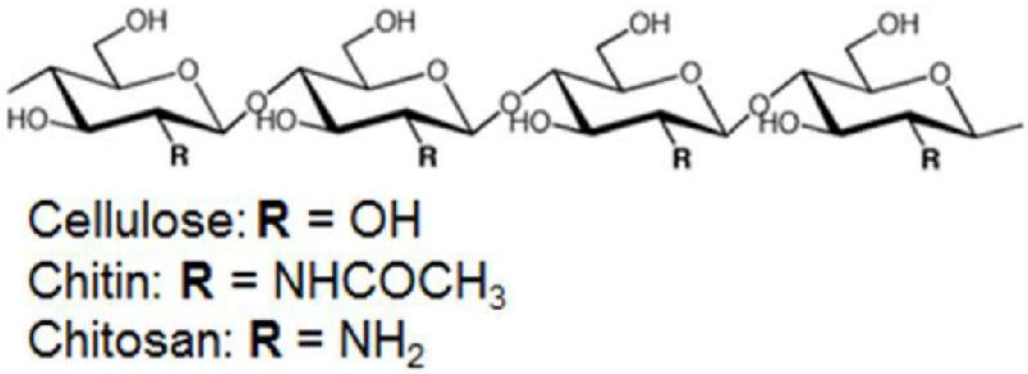

Figure 1: Comparison between the structures of chitin, CS, SA with cellulose [13].

1.3 Alginic acid (AA)

AA, also called algin or alginate, is an anionicpolysaccharide distributed widely in the cell walls of brown algae, as the calcium, magnesium and sodium salts of AA. “Alginate" is the term usually used for the salts of AA, but it can also refer to all the derivatives of AA and AA itself; in some publications the term "algin" is used instead of alginate[14]. Figure 2 shows the molecular structure of AA [15]. In extracted form it absorbs water quickly; it is capable of absorbing water, where through binding with water it forms a viscous gum. It is also a significant component of the bio films produced by the bacterium Pseudomonas aeruginosa, the major pathogen in cystic fibrosis, that confer it a high resistance to antibiotics and killing by macrophages[6]. Its colour ranges from white to yellowish-brown. It is sold in filamentous, granular or powdered forms.

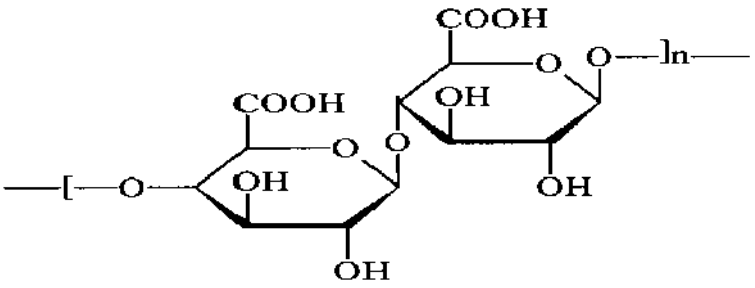

Figure 2: The molecular structure of AA [15].

1.3.1 Structure & bonding of AA

Figure 3 represents the structural bonding between polymeric blocks of AA[15]. AA is a linear copolymer with homopolymeric blocks of (1-4)-linked β-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues (G-blocks), consecutive M-residues (M-blocks) or alternating M and G-residues (MG-blocks) [6].

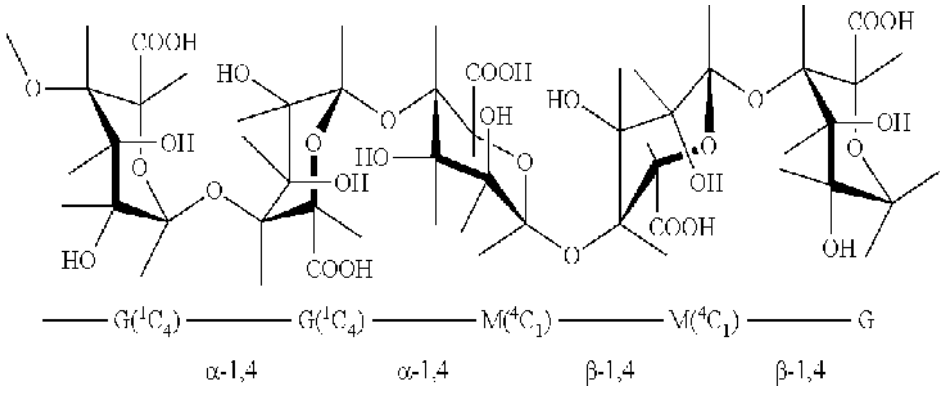

Figure 3: The structural bonding between polymeric blocks of AA [6].

1.3.2 The history of alginate chemistry

Algae are one of the world's oldest life forms, being present in the pre Cambrian era. Alginates are refined from brown seaweeds. Figure 4 shows the photograph [16] of brown seaweeds. A wide variety of brown seaweeds harvested throughout [14] the world to be converted into the raw material commonly known as SA.

Figure 4: Photograph of brown seaweeds [16].

1.3.3 Types of alginate

These algae are classified into four main groups:

- Chlorophyceae, the green algae
- Phaeophyceae, the brown algae
- Rhodophyceae, the red algae
- Cyanophyceae, the blue-green algae

1.3.4 Properties of alginate

Alginates from different species of brown seaweed often have variations in their chemical structure, resulting in different physical properties. For example, some may yield an alginate that gives a strong gel, another weaker gel; some may readily give a cream/white alginate, while others are difficult to gel, and are best used for technical applications where color does not matter [6]. Differences in M/G ratio and block configuration account for the differences in alginate properties and functionality, especially in gelling capability and gel strength. The M/G ratio is dependent upon such factors as the species of seaweed, the part of the seaweed used, the harvest location, and the harvest season. Commercial varieties of alginate are extracted

from seaweed, including the giant kelp Macrocystis pyrifera, Ascophyllum nodosum, and various types of Laminaria Macrocystis pyrifera, the largest species of giant kelp.

It is also produced by two bacterial genera Pseudomonas and Azotobacter, which played a major role in the unravelling of its biosynthesis pathway. BAs are useful for the production of micro- or nano structures suitable for medical applications [7]. As alginate is a linear polymer the viscosity is determined by the molecular weight and the rigidity and extension of the chain. The most common alginate gel used is the CA gel. Alginate will gel with most di and trivalent salts but the calcium gel is really thinly one used in the food industry. PGA (or PGL) is the only, commercially available, chemically modified alginate. PGA is made by contacting a partially neutralized AA with propylene oxide gas under pressure. The propylene oxide reacts exothermically with the AA to form a mixed primary/secondary ester.

1.3.5 Alginate production

The processes for the manufacture of SA from brown seaweeds fall into two categories: 1) Calcium alginate method and, 2) Alginic acid method. The chemistry of the processes used to make SA from brown seaweeds is relatively simple. The difficulties of the processes arise from the physical separations which are required, such as the need to filter slimy residues from viscous solutions or to separate gelatinous precipitates which hold large amounts of liquid within the structure and which resist filtration and centrifugation[6]. alginate can also be produced from bacterial sources.

1.3.6 Preparation of alginate

1.3.6 .1 Extraction of SA

To extract the alginate, the seaweed is broken into pieces and stirred with a hot solution of an alkali, usually sodium carbonate. Over a period of about two hours, the alginate dissolves as SA to give very thick slurry. This slurry also contains the part of the seaweed that does not dissolve, mainly cellulose. This insoluble residue must be removed from the solution. The solution is too thick (viscous) to be filtered and must be diluted with a very large quantity of water. After dilution, the solution is forced through a filter cloth in a filter press. However, the pieces of undissolved residue are very fine and can quickly clog the filter cloth. Therefore, before filtration is started, a filter aid, such as diatomaceous earth, must be added; this holds most of the fine particles away from the surface of the filter cloth and facilitates filtration. However, filter aid is expensive and can make a significant contribution to costs. To reduce the quantity of filter aid needed, some processors force air into the extract as it is being diluted with water (the extract and diluting water are mixed in an in-line mixer into which air is forced).

1.3.6.2 Extraction of CA

The goal of the extraction process is to obtain dry, powdered, SA. The calcium and magnesium salts do not dissolve in water; the sodium salt does. The rationale behind the extraction of alginate from the seaweed is to convert all the alginate salts to the sodium salt, dissolve this in water, and remove the seaweed residue by filtration. The alginate must then be recovered from the aqueous solution. The solution is very dilute and evaporation of the water is not economic. To the SA from the initial extraction solution, a calcium salt is added. This causes CA to form with a fibrous texture; it does not dissolve in water and can be separated from it with relative ease using a metal screen. CA is a water-insoluble, gelatinous, cream-coloured substance that can be created through the addition of aqueous calcium chloride to aqueous sodium alginate.

1.3.6.3 Extraction of bacterial alginate (BA)

Alginate can be produced by a microbial fermentation using bacteria such as Azobacter Vinelandii and Pseudomonas Aeruginosa. These bacteria produce a polysaccharide with a structure resembling alginate, differing only in that there are acetyl groups on a portion of the C_2 and C_3 hydroxyls. It is believed that the acetate groups are associated mainly with the D-mannuronic acid residues. The level of acetylation is variable as is the mannuronic and guluronic acid content. However the level of guluronic acid in the final polymer can be controlled to some extent by altering the level of calcium in the fermentation broth. The sequence structures and acetylation patterns of BA, from different sources, have been studied with 2D COSY proton NMR techniques. The acetyl residues were found to be exclusively associated with the mannuronic acid residues with degrees of acetylation varying from 4-57% one other interesting point noted was that alginate isolated from four different pseudomonas bacteria all showed a complete lack of consecutive guluronic acid segments, a necessary structural feature for the formation of calcium gels.

PGA is produced by taking an AA sample that has been pressed to contain between 30-40% solids and partially neutralise the acid with sodium carbonate to between 5-40% neutralised. The solids are then washed in acetone or propan-2-ol and centrifuged to remove the excess liquid. Finally a fibre with 60-87% solids is obtained. The fibre is then treated with 1, 2 epoxypropane to form an ester. The levels of substitution of over 90% can be produced.

1.3.7 Difference between AA, SA and CA

AA is an anionic polysaccharide shown in figure 2. The empirical formula of SA is $NaC_6H_7O_6$ which is a chemical compound that is the sodium salt of AA. CA, made from SA from which the sodium ion has been removed and replaced with calcium,

has the chemical formula $C_{12}H_{14}CaO_{12}$. Figure 5 describes the structural differences between AA, SA and CA and figure 6 shows the structure of SA [17] [18].

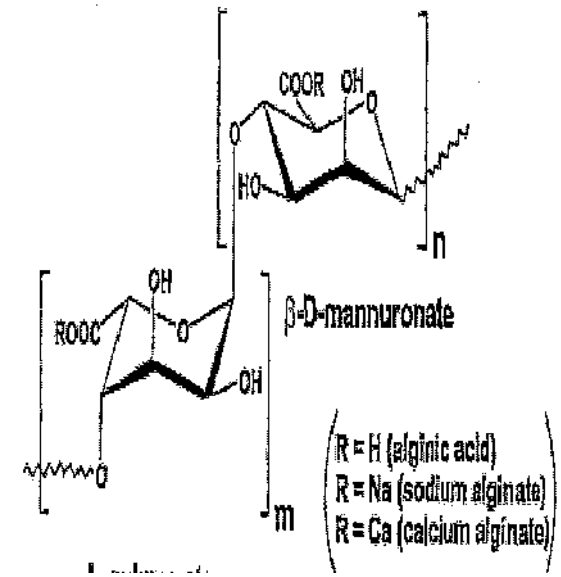

Figure 5: Structural differences between AA, SA and CA [17].

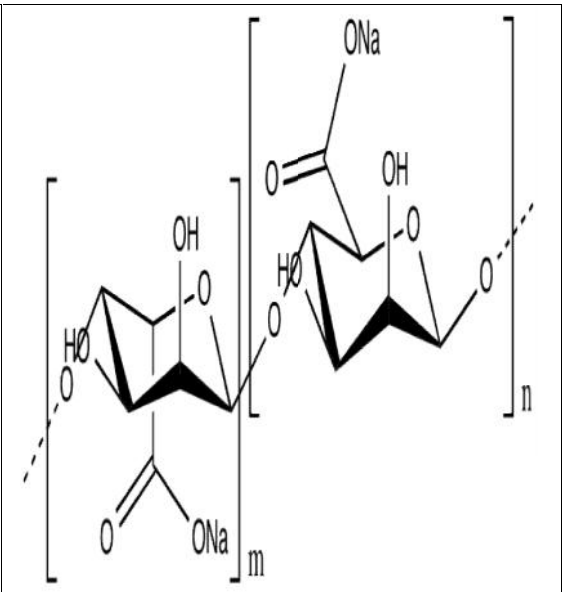

Figure 6: Structure of SA [18].

1.3.8 Solubility of Alginates

The solubility of alginate [17] to water and solvents is illustrated in table 1 & table 2**.** Table 1 represents the solubility of alginate to various types of solvents and table 2 represents thesolubility of alginate to various types of solutions.

- All the alginate salts are insoluble to fats & oils and organic solvents.
- Salts of AA with monovalent cations (Na-salt, K-salt, Ca-salt, NH_4-salt) as well as alginate ester are all soluble to cold & hot water, and generate viscous aqueous solution with long-flow properties.
- AA and CA are water-insoluble.
- CA is also insoluble in ether; slightly soluble in ethanol; slowly soluble in solutions of sodium polyphosphate, sodium carbonate, and substances that combine with calcium ions. SA dissolves slowly in water, forming a viscous solution; insoluble in ethanol and ether.
- Solubility of alginate changes to some extent with the properties of solution (pH, concentration of cation, etc.)
- Care is required when making a solution of low pH or a solution that contains polyvalent cation, because alginate is hard to be soluble in these systems.
- SA, PA and AAL shows a tendency that the viscosity of alginate solution drops in the heavy alkaline system. In table 2, it is also observed that alginate dissolves even in the alkaline system.In the neutral and alkaline system, alginate ester is decomposed and generates alginate salt.

Table 1: Solubility of alginate to various type of solvent

Type of Alginate	**Solubility**		
	Cold & Hot water	**Fats & Oils**	**Organic solvents**
AA	Insoluble	Insoluble	Insoluble
SA	Soluble	Insoluble	Insoluble
PA	Soluble	Insoluble	Insoluble
CA	Insoluble	Insoluble	Insoluble
AAL	Soluble	Insoluble	Insoluble
PGL	Soluble	Insoluble	Insoluble

Table 2: Solubility of alginate to various type of solution

Types of Alginate	**Solubility in acidic solution**	**Solubility in alkaline solution**	**Solubility in solution with polyvalent cation**
	e.g.: Fruit juice, Liquor, Salad dressing, etc.	**e.g. Kansuri**	**e.g.: Hard water, Milk etc.**
AA	Insoluble	Soluble	Insoluble
SA	Insoluble	Soluble	Insoluble
PA	Insoluble	Soluble	Insoluble
CA	Insoluble	Insoluble	Insoluble
AAL	Insoluble	Soluble	Insoluble
PGL	Soluble	Soluble	Soluble

1.3.9 Uses of alginates

- Safety of alginate for food applications is certified by FAO/WHO and Food and Drug Administration, as one of the safest food additives. In production, SA is extracted from brown algae and is the sodium salt of AA. It is highly viscous and is often used as an emulsifier and a gelling agent. These properties give SA a variety of uses in many industries.
- Alginate absorbs water quickly, which makes it useful as an additive in dehydrated products such as slimming aids, and in the manufacture of paper and textiles. It is also used for waterproofing and fireproofing fabrics, in the food industry
- Alginate is used as an ingredient in various pharmaceutical preparations, such as Gaviscon, in which it combines with bicarbonate to inhibit reflux.
- SA is used in reactive dye printing and as a thickener for reactive dyes in textile screen-printing. As a paint and dye thickeners, when SA is added to

paint or dye, it increases the thickness of either substance. This is useful when trying to adjust the consistency of a product without changing its color.

- In plant tissue culture to produce insoluble artificial seeds, for immobilizing enzymes by entrapment, to produce an edible substance incorporated into wound dressings (alginate dressings) as a haemostatic.
- Alginate is used in cosmetics area with several applications with its functionality of thickener and moisture retainer. Alginate helps retaining the color of lipstick on lip surface by forming gel-network.
- Alginic acid is used in pharmaceutical area with several applications. AA is compounded into tablets to accelerate disintegration of tablet for faster release of medicinal component. Alginate forms gel in the high-acidic stomach and protect stomach mucus.
- Alginate has an excellent functionality as a thickening agent, gelling agent, emulsifier, stabilizer, texture-improver (for noodles), to improve the quality of food. Nowadays, based on unique and excellent properties alginate is applied to numerous kinds of food, such as ice cream, jelly, lactic drinks, dressings, instant noodle, beer etc.
- Alginate is used as a binder and thickening agent for pet-food, fish feed, etc.
- Alginate is used for the production of welding rod, as a binder of flux.
- Alginate is used for substrate of color paste when applying patterns to print fabrics, scarf, towel, etc. Use of alginate for printing of cotton, jute, rayon is mandatory. Alginate, a seaweed extract, is safer and easier to be decomposed compared with other substrate for textile printing, and gives easier waste water disposal.
- Alginate used as making spheres. Molecular gastronomy is a method of applying scientific principles to cooking or baking. In 2007, Science Careers published an article on how Spanish Chef Ferran Adrià imitated caviar by mixing SA and fruit juice into a calcium solution. When mixed in the right proportions before the addition to the calcium solution, the substance is found to be in liquid form. When expunged through a syringe, drop by drop into the calcium solution, the liquid forms a gelatin coating and resembles individual caviar.
- Alginate used as a good chelator. A chelator is any substance that removes heavy metal toxins from the bloodstream by binding with them. Heavy metal toxins are found in the bloodstream for a variety of reasons, including diet, exposure to the environment and exposure to materials. In 1966, the National Institutes of Health published findings that SA in specified doses was capable of reducing absorption of the metal strontium. The study found that even though SA effectively reduced strontium absorption, it did not affect the absorption of calcium [18].
- Making homogeneous solution is the most important procedure when obtaining the maximum performance of so-called “alginate”. The negatively charged carboxyl groups in alginates cause the straight alginate chains to repel each other and results in a stable aqueous solution. This aqueous solution has smooth long-flow properties with Newtonian behavior. SA has a strong affinity with water and care is required to achieve a homogeneous aqueous solution. It’s most important to first prepare uniform aqueous solution of alginate before it is utilized as a thickening agent, gelling agent,

etc. Poor dispersion in water will occur if SA is added too rapidly, producing pasty, floury lumps wetted only on the outside. SA has the ability to clump if added to water quickly because of its gelatinous nature. To reduce clumping, either has to add SA slowly while stirring or mix the day before in a blender.

1.4 Chitin and CS

1.4.1 Introduction

Chitin and CS [5] are considerably versatile and promising biomaterials. Chitin is the second most ubiquitous natural polysaccharide after cellulose on earth which is composed of β (1→4)-linked 2-acetamido-2-deoxy-β-D-glucose1 (Figure 6). It is often considered as cellulose derivative, even though it does not occur in organisms producing cellulose. It is structurally identical to cellulose, but it has acetamide groups (−NHCOCH3) at the C-2 positions. Similarly the principle derivative of chitin, CS is a linear polymer of α (1→4)-linked 2-amino-2-deoxy-β-D-glucopyranose and is easily derived by N-deacetylation, to a varying extent that is characterized by the degree of deacetylation, and is consequently a copolymer of N-acetyl glucosamine and glucosamine. Figure 7 shows the structure [19] changes from chitin to CS.

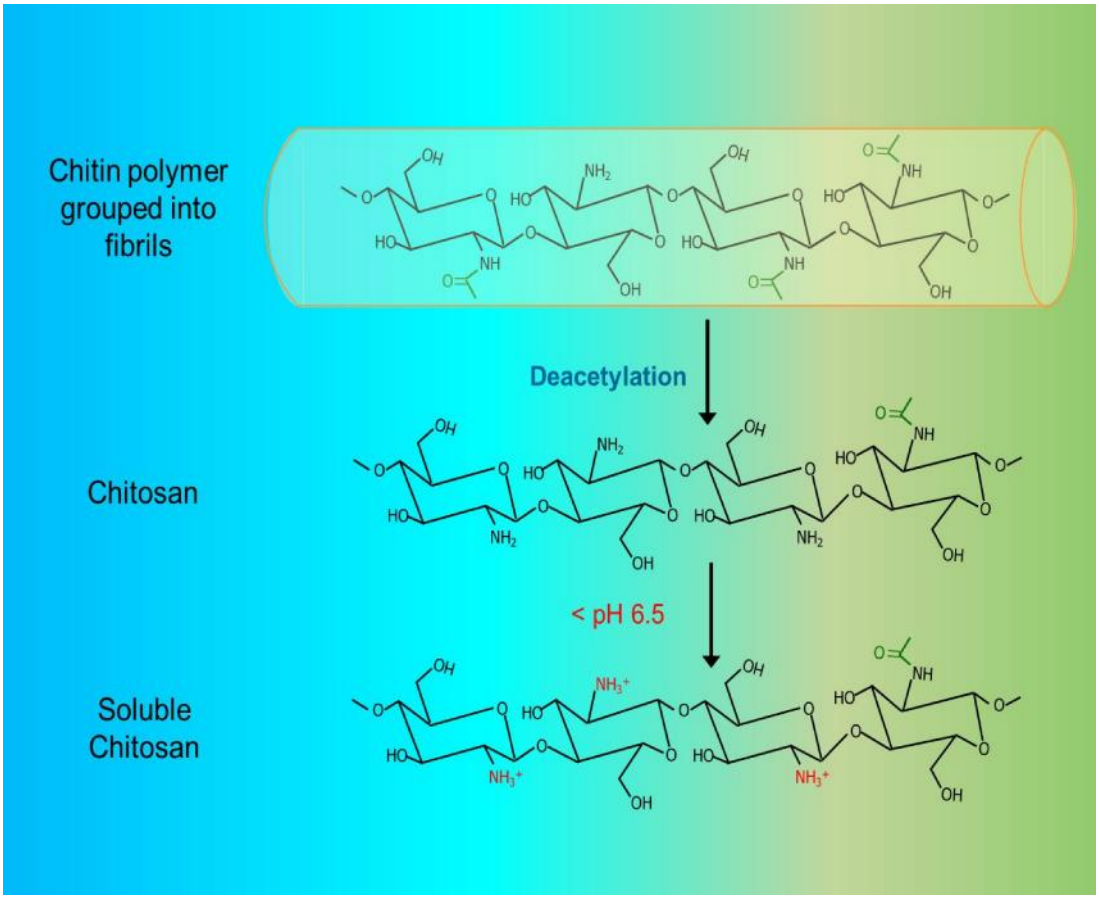

Figure 7: Chitin to CS [19].

CS does have some functional flaws however. Commercial processing to consistently yield a high quality product can be difficult and expensive and it is relatively insoluble in water above pH 6.5. The use of functionalizing reagents to generate substituted amine groups either at, or appended to CS primary amines, will overcome the solubility problem and has been shown to enhance the antibacterial properties of CS. However, further research and investment in this area will in time generate functionalized CS products at the right cost and of sufficient quality and purity to be suitable for the broader market.

The healing properties of chitin have been known for centuries. Healers used apparent the haemostatic properties of chitin materials to treat cuts and abrasions this property in fact coming from a natural degradation product of chitin; namely CS. CS is produced by the natural, chemical or physical hydrolysis of pendant acetyl groups on the polymer chain. The primary amines that are left are positively charged under mildly acidic conditions, rendering CS soluble unlike chitin and providing it with bacteriostatic properties. The potential commercial applications of CS have grown steadily since the late twentieth century, but numbers of patent applications have exploded in the last 10 years.

CS is now sold within products for wound healing, cosmetics and toiletries, nutraceuticals and dietary aids, water treatment and agricultural and horticultural applications and with bio-medical and biotechnological applications for CS becoming one of the strongest, high-value growth markets. The broad utility of CS comes from its physical properties; it is relatively rare in being a cationic natural polymer, it binds metal ions and is film forming, also from its biological properties; it is antibacterial, adheres selectively to biological surfaces (e.g. to tumour cells) and is biodegradable. However, the great commercial potential of CS is due to its chemical properties in that it can be readily degraded to oligomers and monosaccharide building blocks, but can also be readily modified, chemically or enzymically to enhance its natural properties, generate entirely new functional properties or confer CS-like properties onto other chemicals.

Chitin and CS the naturally abundant and renewable polymers have excellent properties such as, biodegradability, bio-compatibility, non-toxicity, and adsorption too. The reaction of CS is considerably more versatile than cellulose due to the presence of $-NH_2$ groups. It is chitin and CS which can readily be derivatized by utilizing the reactivity of the primary amino group and the primary and secondary hydroxyl groups to find applications in diversified areas.

1.4.2 The history of chitin

Chitin and CS are valuable veritable natural materials derived from shells of prawns and crabs. The word “Chitin” comes from the Greek etymology meaning “A Coat Of Mail”. The product was first used by Odier in 1823.Prof Henri Braconnot of France

first discovered chitin in mushrooms in 1811. In 1830s, it was isolated in insects and was named chitin. Prof C. Rouget discovered CS in 1859. In 1930s and 1940s, the polymer attracts considerable attention as evidenced by about 50 patents.

1.4.3 Extraction of CS from prawn shell waste and its properties

Commercial CS is derived from the shells of shrimp and other sea crustaceans, including Pandalus borealis CS is produced commercially by deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans (such as crabs and shrimp) and cell walls of fungi. Figure 8 shows the photograph [20] of washed Prawn Shell.

Figure 8: Photograph of prawn shell [20].

The degree of deacetylation (%DD) can be determined by NMR spectroscopy, and the percentage of DD in commercial CSs ranges from 60 to 100%. On average, the molecular weight of commercially produced CS is between 3800 and 20,000 Daltons. A common method for the synthesis of CS is the deacetylation of chitin using sodium hydroxide in excess as a reagent and water as a solvent. This reaction pathway, when allowed to go to completion (complete deacetylation) yields up to 98% product [2].

1.4.3.1 Properties of chitin and CS

Most of the naturally occurring polysaccharides e.g., cellulose, dextrin, pectin, AA, agar, agarose, and carragenas are natural and acidic in nature, whereas chitin and CS are examples of highly basic polysaccharides. Their properties include solubility in various media, solution, viscosity, polyelectrolyte behavior, polyoxy salt formation, ability to form films, metal chelations, optical, and structural characteristics.

1.4.3.2 Chemical properties of CS

The chemical properties of CS are as follows

- Linear polyamine
- Reactive amino groups
- Reactive hydroxyl groups available
- Chelates many transitional metal ions.

1.4.3.3 Biological properties of CS

The biological properties of CS are as follows

- Biocompatible
- Natural polymer
- Biodegradable to normal body constituents
- Safe and non-toxic
- Binds to mammalian and microbial cells aggre- sively
- Regenerative effect on connective gum tissue
- Acclerates the formation of osteoblast responsible for bone formation
- Hemostatic
- Fungistatic
- Spermicidal
- Antitumor
- Anticholesteremic
- Accelerates bone formation

1.4.4 Applications of chitin and CS

- Due to its physical and chemical properties, CS is being used in a vast array of widely different products and applications, ranging from pharmaceutical and cosmetic products to water treatment and plant protection. In different applications, different properties of CS are required. These properties change with, e.g., degree of acetylation and molecular weight as well.
- Usually organic acids are used as good solvents for cosmetic [7] applications. A natural aminopoly- saccharide, CS can be encompassed in the class of hydrocolloids. However, unlike the most of other hydrocolloids which are polyanions CS is the only natural cationic gum that becomes viscous on being neutralized with acid. It facilitates its interaction with common integuments (skin covers) and hair. Chitin and CS are fungicidal and fungistatic in nature. CS is compatible with lots of biologically active components incorporated in cosmetic products composition. CS or CS-SA composites in the range of 1-10 μ, as well as microcapsules including various hydrophobic substances find a wide application in cosmetics.
- Due to its polycationic nature, CS can be used as flocculating agent. It can also act as chelating agent, and heavy meatls trapper. CS N-benzyl sulphonate derivatives as sorbents for removal of metal ions in acidic medium.

- Biodegradable chitin and CS can strengthen recycled paper and increase the environmental friendliness of packaging and other products. CS is already involved in the manufacture of paper because CS molecules greatly resemble those of cellulose the main constituent of plant walls. It also saves chemical additives and increases output. Lastly the paper produced with CS has a smoother surface and is more resistant to moisture. Among other things, CS is of great value in the production of toilet paper and for wrapping paper and cardboard.
- Derivatives of chitin have been produced and used to impart antistatic and soil repellent characteristics to the textiles [8]
- Use of CS in food industry is well known because it is not toxic for warm-blooded animals. Microcrystalline chitin (MCC) shows good emulsifying properties, superior thickening, and gelling agent for stabilizing foods [9]. It is also used as a dietary fibre in baked foods. The use of MCC solved some of the problems such as, flavor, color, and shelf-life, posed by other sources of fibre. It could be of special importance for manufacturing protein-fortified bread, even without such ingredients as emulsifiers and shortenings. Chitin and CS act as solid support for the entrapment of whole microbial, animal, or plant cell immobilization. Chitin has been used in immobilization of enzymes. In 2016 researchers announced a CS-based plastic wrap that doubles the shelf life of some foods. The plastic also included grape fruit seed extract, which has antibacterial and antifungal properties, and is an antioxidant, antiseptic and anti-viral. The film blocked the transmission of ultraviolet light slowing oxidation and photochemical deterioration. The plastic can use raw ingredients that would otherwise be discarded, and biodegrades once discarded.
- Chitin and CS find wide varieties of applications in chromatographic separations [10]
- Recently, dyes containing CS gels have been used as potential components in lasers and other light-emitting devices LEDs[11] .
- The design of artificial kidney systems has made possible repetitive hemodialysis and the sustaining life of chronic kidney failure patients. CS membranes have been proposed as an artificial kidney membrane because of their suitable permeability and high tensile strength [12]. The most important part of artificial kidney is the semipermeable membrane and so far made from commercial regenerated cellulose and cuprophane. Since the primary action of the cellulose membrane is that of a sieve, there is little selectivity in the separation of two closely related molecules. These novel membranes need to be developed for better control of transport, ease of formability and inherent blood compatibility. A series of membranes prepared from chitin and its derivatives improved dialysis properties. One of the most serious problems of using these artificial membranes is surface induced thrombosis, where heparization of blood is needed to prevent clotting, and people who are liable to internal hemorrhage can be dialysed only at great risk. Hence, these are the most challenging problem still to be resolved in the development of membranes which are inherently blood compatible. From these point of views, CS is hemostatic, i.e., causes clots.

- Tissue engineering is the development and manipulation of laboratory-grown cells, tissues or organs that would replace or support the function of defective or injured parts of the body. The many potential advantages of tissue engineering include the development or revolution of current technology in total hip, knee, cartilage, tendon, and vascular replacement.
- CS is a promising candidate for burn treatment. This is true since CS can form tough, water-absorbent, biocompatible films. CS has replaced the synthetic polymers in opthalmological applications.
- The agricultural and horticultural uses for CS, primarily for plant defense and yield increase, are based on how this glucosamine polymer influences the biochemistry and molecular biology of the plant cell. The cellular targets are the plasma membrane and nuclear chromatin. Subsequent changes occur in cell membranes, chromatin, DNA, calcium, MAP Kinase, oxidative burst, reactive oxygen species, callose pathogenesis-related (PR) genes and phytoalexins.Since 1986, the United States Environmental Protection Agency has regulated CS for agricultural use.
- CS is used as natural biocontrol and elicitor in agriculture, CS is typically used as a natural seed treatment and plant growth enhancer, and as an ecologically friendly biopesticide substance that boosts the innate ability of plants to defend themselves against fungal infections. It is one of the most abundant biodegradable materials in the world.
- CS and chitooligosaccharides (CHOS) are polysaccharides with a broad range of applications, including plant growth promoting activities. Both compounds have also been shown to have metal chelating properties. However, several factors, such as degree of polymerization, degree of deacetylation, pH, temperature, concentration, application method, viscosity and purity, can influence CS and CHOS mode of action. Most applications of CSs and CHOS so far have been in the biomedical industry. But given that there is a great interested to find novel ways to enhance the plant's nutritional value (biofortification) or to modulate the capacity of plants to extract toxic metals from the soil (phytoremediation), a better understanding of CS and CHOS effects in plant systems is warranted [21].

1.4.5 Difference between CS and SA

- CS and alginate are polysaccharides. CS is a polymer of D-glucosamine and N-acetylglucosamine, and alginate is a polymer with repeating unit of β-D-manuronil linked with α-L-guluronil. The lysozyme only recognizes glycosidic linkages between N-acetylglucosamine units. Therefore it is unable to hydrolyze the alginate.
- Lysozymes are enzymes present in plant and human that can hydrolyze the β(1-4) linkages between N-acetylglucosamine and glucosamine in CS and chitin according to the distribution and proportion of N-acetyl group. This enzyme is well described as dependent on N-acetyl degree and then is more active on chitin than CS because chitin has more N-acetyl glucosamine residues.

- CS is basically the deactylated product of chitin. In order to make it soluble, deacetylation is carried out using NaOH. So CS is basically a polymer with random units of D glucosamine and N-acetyl D glucosamine.
- Lysozyme normally cleaves glycosidic bonds between C-1 of N-acetyl muramic and C-4 of N-Acetylglucosamine (which form the repeating units of peptidoglycan) and normally specifically attacks only the bond after identifying N acetyl D- glucosamine. This is the reason why Lysozyme also has capability to break chitin and so CS which is iits deacetylated product. However, alignic acid or alginate is polymer of rare mannuronic acid and guluronic acid linked through 1,4 linkage which cannot be identified by Lysozyme and hence it is stable in presence of this enzyme.
- So, if we can prepare a sample of alginate which has N-acetyl groups on its carboxylic moieties, the resulting polymer could be recognized by lysozyme.
  It means N-acetyl part is only a minor part of the substrate recognition by the enzyme. The one that taken into account the most are the D-glucosamine and the beta 1-4 linkage. If we take a look on how different the monomer of both polysaccharides are, we will see that the amine group of D-glucosamine and carboxyl group of D-mannuronic/L-guluronic acid are located in a completely different location. Carboxyl moiety of the D-mannuronic/L-guluronic acid is actually located on C6 which in D-glucosamine counterpart is hydroxymethyl group (CH_2OH). Lysozyme is a non-specific enzyme like lipase or CYP 450, then lysozyme won't be able hydrolyzes other substrate than CS/chitin. Carboxyl versus amine aside, there are 5 chiral atoms in every monomer, which means every hydroxide/ether group must be in the correct position to be bound by the corresponding amino acid residue inside the binding cavity of lysozyme.

1.5 Plant growth promoters

Plant growth regulators are chemicals applied by a horticulturist to regulate plant growth (figure 9). In plant propagation, cuttings are dipped in a rooting hormone to stimulate root development. In greenhouse production, many potted flowering plants (like poinsettias and Easter lilies) may be treated with plant growth regulators to keep them short. Seedless grapes are treated with plant growth regulators to increase the size of the fruit.

1.5.1 Plant growth factors

The three major functions that are basic to plant growth and development are

- Photosynthesis – The process of capturing light energy and converting it to sugar energy, in the presence of chlorophyll using carbon dioxide and water.
- Respiration – The process of metabolizing (burning) sugars to yield energy for growth, reproduction, and other life processes.
- Transpiration **–** The loss of water vapor through the stomata of leaves

1.5.2 Signals and regulators on plant

Plants produce hormones and other growth regulators which act to signal a physiological response in their tissues. They also produce compounds such as phytochrome that are sensitive to light and which serve to trigger growth or development in response to environmental signals. Figure 9 shows the abnormal growth of the right plant due to the lack of hormone auxin [22].

Figure 9: Photograph of the effect on plant growth due to the lack of the plant hormone auxin (right) [22].

1.6 Plant hormones

Plant hormones [23], known as plant growth regulators (PGRs) or phytohormones, are chemicals that regulate a plant's growth. According to a standard animal definition, hormones are signal molecules produced at specific locations that occur in very low concentrations and cause altered processes in target cells at other locations. Unlike animals, plants lack specific hormone-producing tissues or organs. Plant hormones are often not transported to other parts of the plant and production is not limited to specific locations.

Plant hormones are chemicals that in small amounts promote and influence the growth, development and differentiation of cells and tissues. Hormones are vital to plant growth; affecting processes in plants from flowering to seed development, dormancy, and germination. They regulate which tissues grow upwards and which grow downwards, leaf formation and stem growth, fruit development and ripening, as well as leaf abscission and even plant death. The most important plant hormones are

abscissic acid, auxins, ethylene, gibberellins, and cytokinins, though there are many other substances that serve to regulate plant physiology.

1.6.1 Hormones influence process in plants

Figure 10a shows the photograph [22] of the effect of auxin on plants growth. In plant, different hormones influence in different way [23, 24].

- Auxins produced in the terminal buds suppress the growth of side buds and stimulate root growth. They also affect cell elongation (tropism), apical dominance, and fruit drop or retention.
- Gibberellins affect
- The rate of cell division
- Flowering
- Increase in size of leaves and fruits
- Seed and bud dormancy

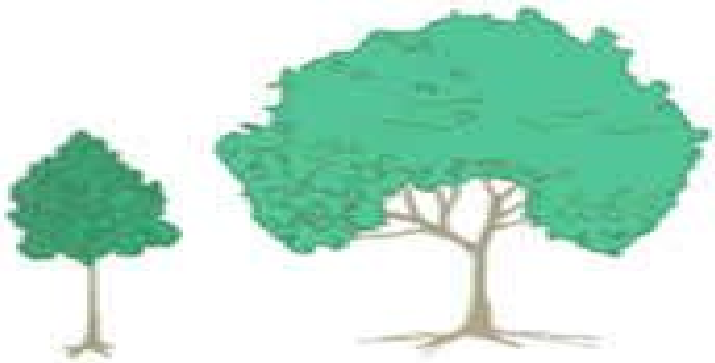

Figure 10a: Photograph of the effect of auxin on plants growth [22]

Auxins produced in the rapidly growing terminal buds suppress growth of side buds, giving a young tree a more upright form. As growth rates slow with age, reduction in apical dominance gives the maturing tree a more rounded crown.

- Induction of growth at lower temperatures (used to green up lawns 2 to 3 weeks earlier)
- Cytokinins promote cell division, and influence cell differentiation and aging of leaves.
- Abscisic acid is considered the “stress” hormone. It inhibits the effects of other hormones to reduce growth during times of plant stress.

1.6.2 Hormone influence procedure on pruning in plants

Understanding hormones is key to proper pruning. Auxin produced in the terminal buds suppresses growth of side buds and stimulates root growth. Gibberellinsproduced in the root growing tips stimulate shoot growth [23]. Figure 10b

shows the tree balances canopy growth with root growth with the levels of auxins and gibberellins [22].

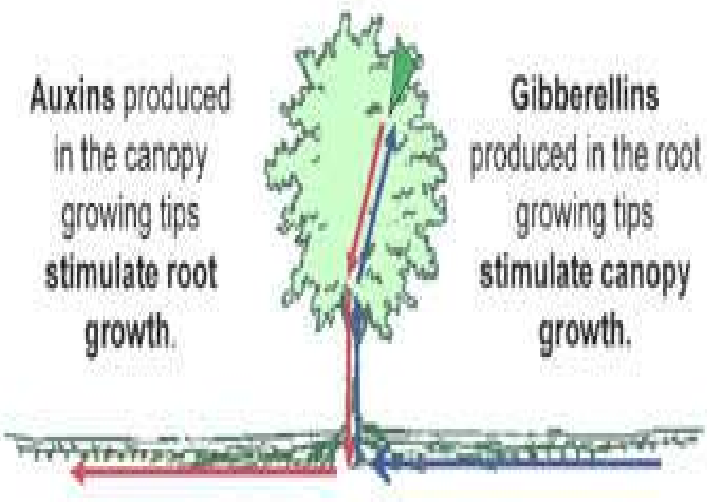

Figure 10(b): Photograph of the effect of auxin on plants' root growth [22].

Pruning a newly planted tree removes the auxin, slowing root regeneration [23]. Heading cuts (removal of a branch tip) releases the apical dominance caused by auxins from the terminal bud. This allows side shoots to develop and the branch becomes bushier. On the other hand, thinning cuts remove a branch back to the branch union (crotch). This type of cut opens the plant to more light. Most pruning should be limited to thinning cuts shown in figure 11. In left of this figure a heading cut releases apical dominance and the branch becomes denser as the lateral buds begin to grow. In the right of this figure a thinning cut removes a branch back at a branch union (crotch), opening the plant for better light penetration. Thinning cuts promote an open growth habit by redirecting sugars to the terminal shoots [24].

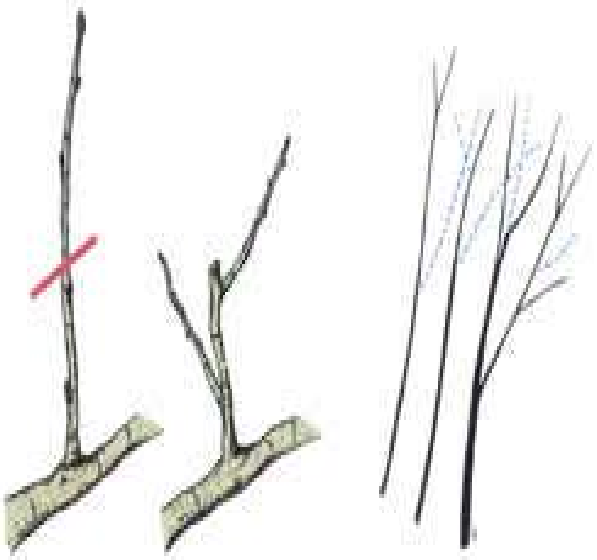

Figure 11: Photograph of the effect of auxins on pruning [24].

1.6.3 Tropisms and nastic movements of plants

Plants may respond both to directional and non-directional stimuli. A response to a directional stimulus, such as gravity or sunlight, is called a tropism. A response to a nondirectional stimulus, such as temperature or humidity, is a nastic movement [25].

Tropisms in plants are the result of differential cell growth, in which the cells on one side of the plant elongates more than those on the other side, causing the part to bend toward the side with less growth [25]. Among the common tropisms seen in plants is phototropism, the bending of the plant toward a source of light. Phototropism allows the plant to maximize light exposure in plants which require additional light for photosynthesis, or to minimize it in plants subjected to intense light and heat. Geotropism allows the roots of a plant to determine the direction of gravity and grow downwards. Tropisms generally result from an interaction between the environment and production of one or more plant hormones.

Nastic movements results from differential cell growth (e.g. epinasty and hiponasty), or from changes in turgor pressure within plant tissues (e.g., nyctinasty), which may occur rapidly. A familiar example is thigmonasty (response to touch) in the Venus fly trap, a carnivorous plant [25]. The traps consist of modified leaf blades which bear sensitive trigger hairs. When the hairs are touched by an insect or other animal, the leaf folds shut. This mechanism allows the plant to trap and digest small insects for additional nutrients. Although the trap is rapidly shut by changes in internal cell pressures, the leaf must grow slowly to reset for a second opportunity to trap insects.

1.6.4 Hormone influence on tropism and geotropism in plants

Tropism controls the direction of plant growth. Auxins play a key role in tropism and geotropism. Photograph [25] of the effect of auxins on tropism of plants growth represents in figure 12.

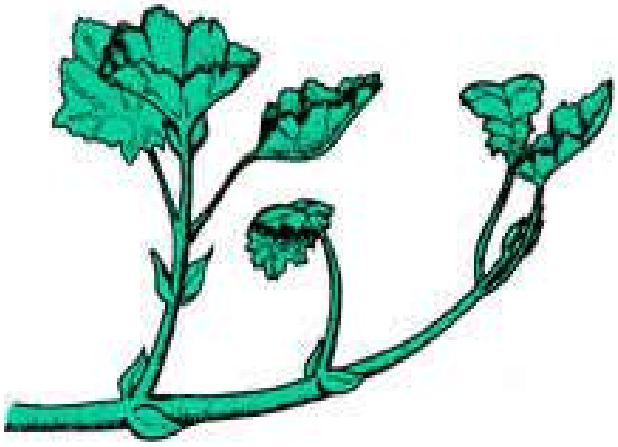

Figure 12: Photograph of the effect of auxins on tropism [25].

Geotropism [25] occurs under the influence of gravity. Auxins accumulate in the lower side of a horizontal stem, causing cells to enlarge faster, turning the stem upright shown in figure 13.

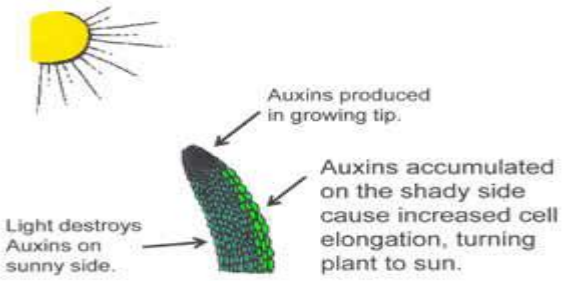

Figure 13: Photograph of the effect of auxins on geotropism [25].

1.7 Fertilizer

A fertilizer is any material of natural or synthetic origin (other than liming materials) that is applied to soils or to plant tissues (usually leaves) to supply one or more plant nutrients essential to the growth of plants. Conservative estimates report 30 to 50% of crop yields are attributed to natural or synthetic commercial fertilizer [26]. Fertilizers enhance the growth of plants. This goal is met in two ways, the traditional one being additives that provide nutrients. The second mode by which some fertilizers act is to enhance the effectiveness of the soil by modifying its water retention and aeration.

The nutrients required for healthy plant life are classified according to the elements, but the elements are not used as fertilizers. Instead compounds containing these elements are the basis of fertilizers [26]. The macronutrients are consumed in larger quantities and are present in plant tissue in quantities from 0.15% to 6.0% on a dry matter (DM) (0% moisture) basis. Plants are made up of four main elements: hydrogen, oxygen, carbon, and nitrogen. Carbon, hydrogen and oxygen are widely available as water and carbon dioxide. Although nitrogen makes up most of the atmosphere, it is in a form that is unavailable to plants. Nitrogen is the most important fertilizer since nitrogen is present in proteins, DNA and other components (e.g., chlorophyll). To be nutritious to plants, nitrogen must be made available in a "fixed" form. Only some bacteria and their host plants (notably legumes) can fix atmospheric nitrogen (N_2) by converting it to ammonia. Phosphate is required for the production of DNA and ATP, the main energy carrier in cells, as well as certain lipids.

Micronutrients are consumed in smaller quantities and are present in plant tissue on the order of parts-per-million (ppm), ranging from 0.15 to 400 ppm DM, or less than 0.04% DM. These elements are often present at the active sites of enzymes

that carry out the plant's metabolism. Because these elements enable catalysts (enzymes) their impact far exceeds their weight percentage.

1.7.1 Types of fertilizer

Fertilizer refers to any compound that contains one or more chemical elements, organic or inorganic, natural or synthetic, that is placed on or incorporated into the soil or applied to directly onto plants to achieve normal growth. The main supply sources of plant nutrients include organic manures. The chemical fertilizers can be broadly classified into: nitrogen, phosphorus, and potassium fertilizers.

A straight fertilizer contains only one of the nutrients. A compound fertilizer contains two or more nutrients.
A complex fertilizer that is formed by mixing ingredients that react chemically, as opposed to a mechanical mixture of two or more fertilizers.
A low analysis fertilizer product contains a low percentage of nutrients, usually 30 per cent or less and a high analysis fertilizer contains more than 30 per cent.
Compound fertilizers, which contain N, P, and K, can often be produced by mixing straight fertilizers. In some cases, chemical reactions occur between the two or more components. For example monoammonium and diammonium phosphates, which provide plants with both N and P, are produced by neutralizing phosphoric acid (from phosphate rock) and ammonia (from a Haber facility):

$NH_3 + H_3PO_4 \rightarrow (NH_4)H_2PO_4$----------(Equation 1)

$2\ NH_3 + H_3PO_4 \rightarrow (NH_4)_2HPO_4$_____ (Equation 2)

1.7.2 Organic fertilizers

The main "organic fertilizers" are, in ranked order, peat, animal wastes, plant wastes from agriculture, and sewage sludge. In terms of volume, peat is the most widely used organic fertilizer. This immature form of coal confers no nutritional value to the plants, but improves the soil by aeration and absorbing water. Animal sources include the products of the slaughter of animals. Bloodmeal, bone meal, hides, hoofs, and horns are typical components [27]. Organic fertilizer usually contains fewer nutrients, but offer other advantages as well as appealing to environmentally friendly users.

1.7.3 Biofertilizer

Bio fertilizeris a substance which contains living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant. Bio-fertilizers add nutrients through the natural processes

of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances [27]. Bio-fertilizers can be expected to reduce the use of chemical fertilizers and pesticides. The microorganisms in bio-fertilizers restore the soil's natural nutrient cycle and build soil organic matter. Through the use of bio-fertilizers, healthy plants can be grown, while enhancing the sustainability and the health of the soil. Since they play several roles, a preferred scientific term for such beneficial bacteria is "plant-growth promoting rhizobacteria"[29]. Therefore, they are extremely advantageous in enriching soil fertility and fulfilling plant nutrient requirements by supplying the organic nutrients through microorganism and their byproducts. Hence, bio-fertilizers do not contain any chemicals which are harmful to the living soil.

Bio-fertilizers provide eco-friendly organic agro-input and are more cost-effective than chemical fertilizers. Bio-fertilizers such as Rhizobium, Azotobacter, Azospirilium and blue green algae have been in use a long time. Rhizobiuminoculant is used for leguminous crops. Azotobacter can be used with crops like wheat, maize, mustard, cotton, potato and other vegetable crops. Azospirillum inoculations are recommended mainly for sorghum, millets, maize, sugarcane and wheat. Blue green algae belonging to a general cyanobacteriagenus, Nostoc or Anabaena or Tolypothrix or Aulosira, fix atmospheric nitrogen and are used as inoculations for paddy crop grown both under upland and low-land conditions. Anabaena in association with water fern Azolla contributes nitrogen up to 60 kg/ha/season and also enriches soils with organic matter.

Other types of bacteria, so-called phosphate-solubilizing bacteria, such as Pantoea agglomerans strain P5 or Pseudomonas putida strain P13, are able to solubilize the insoluble phosphate from organic and inorganic phosphate sources. In fact, due to immobilization of phosphate by mineral ions such as Fe, Al and Ca or organic acids, the rate of available phosphate (P_i) in soil is well below plant needs. In addition, chemical P_i fertilizers are also immobilized in the soil, immediately, so that less than 20 percent of added fertilizer is absorbed by plants. Therefore, reduction in P_i resources, on one hand, and environmental pollutions resulting from both production and applications of chemical P_i fertilizer, on the other hand, have already demanded the use of new generation of phosphate fertilizers globally known as phosphate-solubilizing bacteria or phosphate bio-fertilizers [26,27].

1.7.4 Benefits of biofertilizer

A bio-fertilizer provides the following benefits

Since a bio-fertilizer is technically living, it can symbiotically associate with plant roots. Involved microorganisms could readily and safely convert complex organic material in simple compounds, so that plants are easily taken up. Microorganism function is in long duration, causing improvement of the soil fertility. It maintains

the natural habitat of the soil. It increases crop yield by 20-30%, replaces chemical nitrogen and phosphorus by 25%, and stimulates plant growth. It can also provide protection against drought and some soil-borne diseases. Bio-fertilizers are cost-effective relative to chemical fertilizers. They have lower manufacturing costs, especially regarding nitrogen and phosphorus use. Some important groups of Bio-fertilizers Azolla-Anabena symbiosis: Azolla is a small, eukaryotic, aquatic fern having global distribution.Prokaryotic blue green algae Anabena azolla resides in its leaves as a symbiont. Azolla is an alternative nitrogen source. This association has gained wide interest because of its potential use as an alternative to chemical fertilizers. Rhizobium: Symbiotic nitrogen fixation by rhizobium with legumes contributes substantially to total nitrogen fixation. Rhizobium inoculation is a well-known agronomic practice to ensure adequate nitrogen.

1.7.5 Environmental physiology of biofertilizer

Paradoxically, the subdiscipline of environmental physiology is on the one hand a recent field of study in plant ecology and on the other hand one of the oldest [27]. Environmental physiology is the preferred name of the subdiscipline among plant physiologists, but it goes by a number of other names in the applied sciences. It is roughly synonymous with ecophysiology, crop ecology, horticulture and agronomy. The particular name applied to the subdiscipline is specific to the viewpoint and goals of research. Whatever name is applied, it deals with the ways in which plants respond to their environment and so overlaps with the field of ecology.

Environmental physiologists examine plant response to physical factors such as radiation (including light and ultraviolet radiation), temperature, fire, and wind. Of particular importance are water relations (which can be measured with the Pressure bomb) and the stress of drought or inundation, exchange of gases with the atmosphere, as well as the cycling of nutrients such as nitrogen and carbon. Environmental physiologists also examine plant response to biological factors. This includes not only negative interactions, such as competition, herbivory, disease and parasitism, but also positive interactions, such as mutualism and pollination.

1.8 Essential plant nutrients for crop fertilization

Proper nutrition is essential for satisfactory crop growth and production. The use of soil tests can help to determine the status of plant available nutrients to develop fertilizer recommendations to achieve optimum crop production. The profit potential for farmers depends on producing enough crops per acre to keep production costs below the selling price. Efficient application of the correct types and amounts of fertilizers for the supply of the nutrients is an important part of achieving profitable yields. There are at least 16 elements known to be essential for plant growth (table 3) [28].

Table 3: Essential plant nutrients

Supplied from air and water	**Supplied from soil and fertilizer sources**	
	Macronutrients	**Micronutrients**
Carbon (C)	Nitrogen (N)	Zinc (Z)
Hydrogen (H)	Phosphorous (P)	Copper (Cu)
Oxygen (O)	Potassium (K)	Iron (Fe)
	Sulphur (S)	Maganese (M)
	Calcium (Ca)	Boron (B)
	Magnesium (Mg)	Chlorine (Cl)
		Molybdenum (Mo)
		Cobalt (Co)

Form of uptake of the macronutrients elements by the plant are given in table 4.

Table 4: Form of uptake of the macronutrients elements by the plant

Element	**Form of uptake**	**Produce**
Nitrogen	NO_3^-, NH_4^+	Nucleic acids, proteins, hormones, etc.
Oxygen	O_2 H_2O	Cellulose, starch, other organic compounds
Carbon	CO_2	Cellulose, starch, other organic compounds
Hydrogen	H_2O	Cellulose, starch, other organic compounds
Potassium	K^+	Cofactor in protein synthesis, water balance, etc.
Calcium	Ca^{2+}	Membrane synthesis and stabilization
Magnesium	Mg^{2+}	Element essential for chlorophyll
Phosphorus	$H_2PO_4^-$	Nucleic acids, phospholipids, ATP
Sulfur	SO_4^{2-}	Constituent of proteins

There are six primary nutrients that plants require most. Plants get the first three—carbon, hydrogen and oxygen from air and water. The other three are nitrogen, phosphorus and potassium. Form of uptake of the macronutrients elements by the plant are given below in table 4.

Carbon (C), hydrogen (H), and oxygen (O) are derived from carbon dioxide (CO_2) and water (H_2O). Nitrogen (N), phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), boron (B), chlorine(Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo) and zinc (Zn) are normally derived from the soil in the form of inorganic salts. 94 to 99.5 per cent of fresh plant material is made up of carbon, hydrogen and oxygen. The other nutrients make up the remaining 0.5 to 6.0%. Macronutrients refer to those elements that are used in relatively large amounts, whereas micronutrients refer to those elements [28] that are required in relatively small amounts.

Nitrogen helps plants make the proteins they need to produce new tissues. In nature, nitrogen is often in short supply so plants have evolved to take up as much nitrogen as possible, even if it means not taking up other necessary elements. If too much nitrogen is available, the plant may grow abundant foliage but not produce fruit or flowers. Growth may actually be stunted because the plant isn't absorbing enough of the other elements it needs.

Phosphorus stimulates root growth, helps the plant set buds and flowers, improves vitality and increases seed size. It does this by helping transfer energy from one part of the plant to another. To absorb phosphorus, most plants require a soil pH of 6.5 to 6.8. Organic matter and the activity of soil organisms also increase the availability of phosphorus.

Potassium improves overall vigor of the plant. It helps the plants make carbohydrates and provides disease resistance. It also helps regulate metabolic activities.

Calcium is used by plants in cell membranes, at their growing points and to neutralize toxic materials. In addition, calcium improves soil structure and helps bind organic and inorganic particles together.

Magnesium is the only metallic component of chlorophyll. Without it, plants can't process sunlight.

Sulfur is a component of many proteins.

Finally, there are eight elements that plants need in tiny amounts. These are called micronutrients and include boron, copper and iron shown in table 5 [28].

Table 5: Form of uptake of the micronutrients elements by the plant

Element	Form of uptake	Produce
Chlorine	Cl^-	Photosystem II and stomata function
Iron	Fe^{2+}, Fe^{3+}	Chlorophyll formation
Boron	HBO_3	Crosslinking pectin
Manganese	Mn^{2+}	Activity of some enzymes
Zinc	Zn^{2+}	Involved in the synthesis of enzymes and chlorophyll
Copper	Cu^+	Enzymes for lignin synthesis
Molybdenum	MoO_4^{2-}	Nitrogen fixation, reduction of nitrates
Nickel	Ni^{2+}	Enzymatic cofactor in the metabolism of nitrogen compounds

Plants need to be fertilized because most soil does not provide the essential nutrients required for optimum growth. All 13 elements must be present in the soil for plant use, in varying degrees of availability, to ensure both the immediate and long term needs of the crop [28]. Some of the commonly used terms to describe levels of nutrient elements in plants include:

Deficient When an essential element is at a low concentration that severely limits yield and produces more or less distinct deficiency symptoms. Extreme deficiencies will lead to death of the plant.

Insufficient When the level of an essential plant nutrient is below that required for optimum yields or when there is an imbalance with another nutrient. Symptoms of this condition are seldom evident.

Sufficient When the concentration of an essential nutrient is present in adequate amounts for optimum crop growth.

Excessive When the concentration of an essential plant nutrient is sufficiently high to result in a corresponding shortage of another nutrient.

Toxic When the concentration of either essential or other elements is sufficiently high to reduce plant growth severely. Severe toxicity will result in death of plants.

1.8.1 Organic and synthetic fertilizers

Organic fertilizers are made from naturally occurring mineral deposits and organic material, such as bone or plant meal or composted manure. Synthetic fertilizers are made by chemically processing raw materials. Organic and synthetic fertilizers provide nutrients in different ways.

In general, the nutrients in organic fertilizers are not water-soluble and are released to the plants slowly over a period of months or even years. For this reason, organic fertilizers are best applied in the fall so the nutrients will be available in the spring. These organic fertilizers stimulate beneficial soil microorganisms and improve the structure of the soil [28]. Soil microbes play an important role in converting organic fertilizers into soluble nutrients that can be absorbed by your plants. In most cases, organic fertilizers and compost will provide all the secondary and micronutrients for plants need.

Synthetic fertilizers are water-soluble and can be taken up by the plant almost immediately. In fact applying too much synthetic fertilizer can "burn" foliage and damage the plants. Synthetic fertilizers give plants a quick boost but does little to improve soil texture, stimulate soil life, or improve the soil's long-term fertility. Because synthetic fertilizers are highly water-soluble, they can also leach out into streams and ponds. Synthetic fertilizers do have some advantages in early spring. Because they are water-soluble, they are available to plants even when the soil is still cold and soil microbes are inactive. For this reason, some organically-based fertilizers (such as PHC all-purpose fertilizer), also contain small amounts of synthetic fertilizers to ensure the availability of nutrients.For the long-term health of garden, feeding of plants by building the soil with organic fertilizers and compost is best. This will give the soil that is rich in organic matter and teeming with microbial life.

Foliar fertilizers are applied directly to leaves. The method is almost invariably used to apply water-soluble straight nitrogen fertilizers and used especially for high value crops such as fruits. Plants can absorb nutrients eight to 20 times more efficiently through their leaf surfaces than through their roots. As a result, spraying foliage with liquid nutrients can produce remarkable yields. For best results, would have to spray plants during their critical growth stages such as transplanting time, blooming time.

1.9 Plant disease

Economically, one of the most important areas of research in environmental physiology is that of phytopathology, the study of diseases in plants and the manner in which plants resist or cope with infection [29]. Plants are susceptible to the same kinds of disease organisms as animals, including viruses [30], bacteria, and fungi, as well as physical invasion by insects and roundworms. The biology of plants differs with animals, their symptoms and responses are quite different. In some cases, a plant can

simply shed infected leaves or flowers to prevent the spread of disease, in a process called abscission. Most animals do not have this option as a means of controlling disease. Plant diseases organisms themselves also differ from those causing disease in animals because plants cannot usually spread infection through casual physical contact. Plant pathogens tend to spread via spores or are carried by animal vectors. Figure14 shows the life cicle [31] of the black rot pathogen and figure 15 shows the photograph [32] of powdery mildew on crops' leaves.

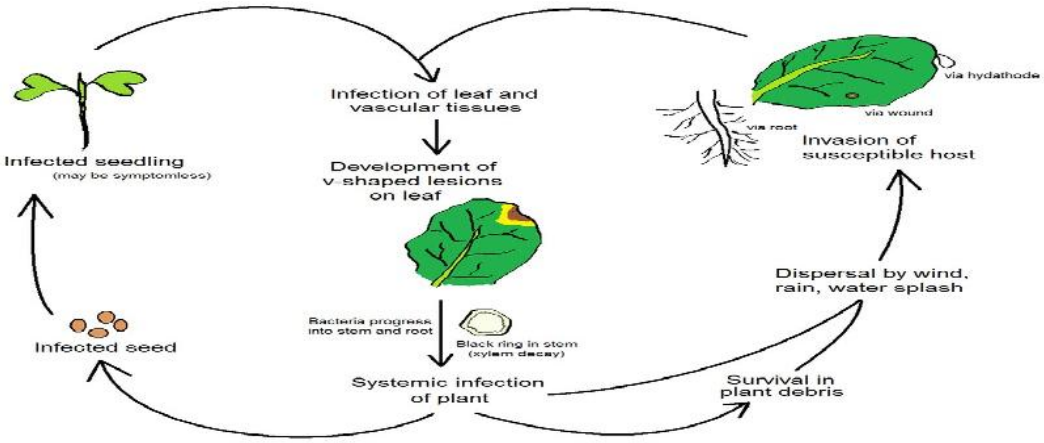

Figure14: Life cicle of the black rot pathogen [31].

Figure 15: Photograph of powdery mildew on crops' leaves [32].

One of the most important advances in the control of plant disease was the discovery of Bordeaux mixture in the nineteenth century. The mixture is the first known fungicide and is a combination of copper sulfate and lime. Application of the mixture served to inhibit the growth of downy mildew that threatened to seriously damage the French wine industry. Most bacteria that are associated with plants are actually saprotrophic and do no harm to the plant itself. However, a small number,

around 100 known species, are able to cause disease. Bacterial diseases are much more prevalent in subtropical and tropical regions of the world.

1.10 Gamma (γ) ray induced polymerization

Gamma ray is a photon of penetrating electromagnetic radiation (gamma radiation) emitted from an atomic nucleus. A photon emitted by an electron as a result of internal conversion. electromagnetic radiation with wavelengths shorter than approximately one tenth of a nanometer [33].

Gamma rays denoted by the lower-case Greek letter gamma (**γ**), are penetrating electromagnetic radiation of a kind arising from the radioactive decay of atomic nuclei. It consists of photons in the highest observed range of photon energy. Paul Villard, a French chemist and physicist, discovered gamma radiation in 1900 while studying radiation emitted by radium. In 1903, Ernest Rutherford named these radiation gamma rays. Rutherford had previously discovered two other types of radioactive decay, which he named alpha rays and beta rays. The decay of an atomic nucleus from a high energy state to a lower energy state, a process called gamma decay, produces gamma radiation. Gamma rays ionize atoms (they are ionizing radiation), and are thus biologically hazardous. Natural sources of gamma rays on Earth are observed in the gamma decay of radionuclides and secondary radiation from atmospheric interactions with cosmic ray particles. There are rare terrestrial natural sources, such as lightning strikes and terrestrial gamma-ray flashes, which produce gamma rays not of a nuclear origin. Additionally, gamma rays are produced by a number of astronomical processes in which very high-energy electrons are produced, that in turn cause secondary gamma rays via bremsstrahlung, inverse compton scattering, and synchrotron radiation. However, a large fraction of such astronomical gamma rays are screened by Earth's atmosphere and can only be detected by spacecraft. Gamma rays are produced by nuclear fusion in stars including the Sun (such as the CNO cycle), but are absorbed or in elastically scattered by the stellar material, reducing their energy, before escaping and are not observable from Earth as gamma rays. Gamma rays typically have energies above 100 keV, and therefore have frequencies above 10 exahertz (or $>10^{19}$ Hz) and wavelengths less than 10 picometers (10^{-11} m), which is less than the diameter of an atom. However, this is not a strict definition, but rather only a rule-of-thumb description for natural processes. Electromagnetic radiation from radioactive decay of atomic nuclei is referred to as "gamma rays" no matter its energy, so that there is no lower limit to gamma energy derived from radioactive decay. This radiation commonly has energy of a few hundred keV, and almost always less than 10 MeV. In astronomy, gamma rays are defined by their energy, and no production process needs to be specified. Solar flares emit gamma rays from nuclear interactions, such as the 2.223 MeV line of neutron capture, but also continuum extending to GeV energies. The energies of gamma rays from more distant and powerful astronomical sources range even higher, to over 10 TeV, an energy far too large to result from radioactive decay.A notable example is

the extremely powerful bursts of high-energy radiation referred to as long duration gamma-ray bursts, of energies higher than can be produced by radioactive decay. These bursts of gamma rays are thought to be due to the collapse of stars called hypernovae.Many common depictions of the electromagnetic spectrum show gamma rays as higher in energy (hence are higher in frequency and smaller in wavelength) than X-rays. This historically allowed a clear distinction between X-rays and gamma rays. Today, the research literature often describes photons depending on their source. While astronomers usually hold to the historical convention (often the source or production mechanism of the radiation is unknown), the physics literature often uses the term historically associated with the method of production [33]. For example, one group of scientists might describe a 1 MeV photon as a gamma ray, while another group use the term X-ray. Figure 16 shows the illustration of an emission of a gamma ray (γ) from an atomic nucleus. Figure 17 shows the common radical mechanism with initiator showing initiation of radical formation by an initiator (initiation), and then propagation of the free radical connecting monomer units (propagation). Reaction finishes with termination, in which two radical chains combine (termination).

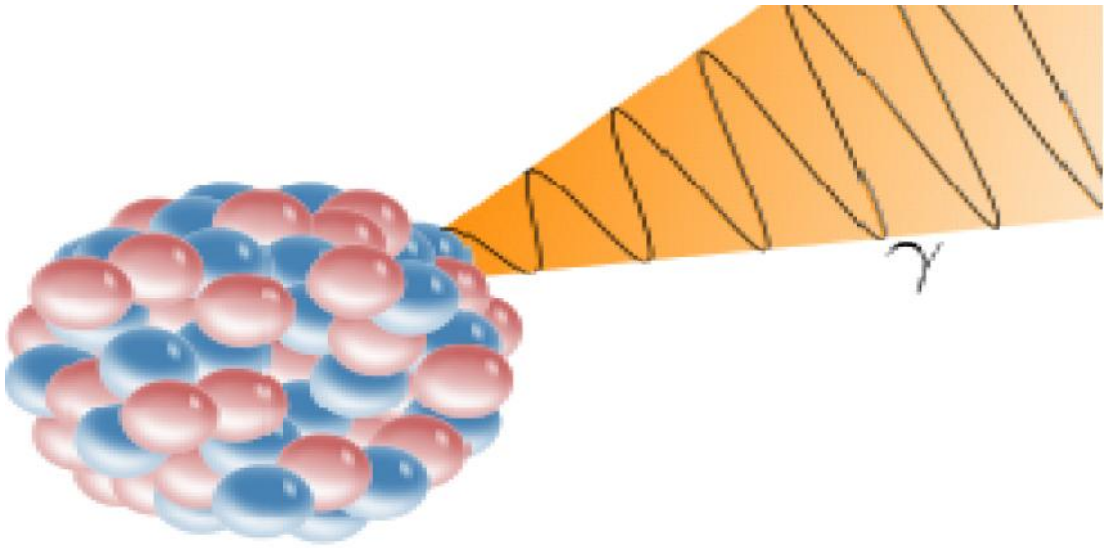

Figure 16: Illustration of an emission of a gamma ray (γ) from an atomic nucleus [34].

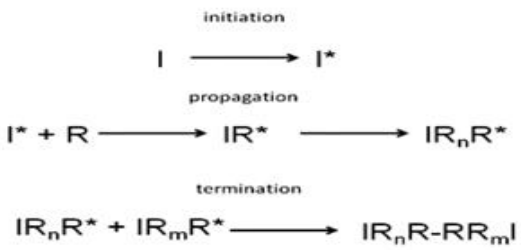

Figure 17 : Scheme for gamma ray induced polymerization [34].

Over the last century, polymers have become prevalent in many commercial products that humans use in their daily lives. The ability to manufacture polymers cheaply and efficiently has played a significant role in the advancement of plastics and their replacement of many metal and ceramic products. While polymers are simply long chains composed of repeating monomer units, the ability to tailor the properties of polymers by give them a variety of engineering uses and scientific uses. One of the significant advantages to using polymers is their relatively low melting point compared to ceramics and metals, making them much more easily shaped into useful technology. However, the exact properties that lead to the useful properties of this polymer are controlled on the molecular level, in which the monomer units are assembled. Reactions that combine these monomer units are commonly referred to as polymerization reactions, and many different mechanisms are used to link them into polymers. One of the more common mechanisms used in polymer chemistry is radical chain polymerization (also sometimes referred to as a living polymerization).

1.10.1 Applications of gamma radiation

Recently, some polymerization reactions have been demonstrated using γ-ray irradiation. One such reaction is the polymerization of methacrylates for metal ion absorption. These polymers can be effective absorbers of toxic metal ions, such as Cr^{6+} and Pb^{2+} from aqueous solutions, making them useful for water purification. The mechanism for the reaction is shown in figure 16. The reaction is carried out at a dose of 20 kGy at 0.5 kGy/hr using a Co^{60} radiation source, which was previously shown to be the optimal conditions for the reaction [34].

1.10.2 Irradiated CS as plant growth promoter

CS undergoes predominantly chain scission when exposed to ionizing radiation which results in production of low molecular weight products named oligomers or oligosaccharides. These oligomers are mobile and easy for uptake by the plant. Radiation has also been shown no effect on the functional groups ($-NH_2$) of CS but instead enhance the anti-microbial activity of the oligosaccharides that improved the plant resistivity towards fungi attacked. These oligomers have potential application in agriculture as plant growth promoter [35]. They studied hydroponic systems for some specific type of crops. The growth promotion activity of radiation depolymerized CS has been investigated in wheat (Triticum eastivum) and chickpea (Cicer arietinum). The growth promotion effect on the seedling growth of two crops was studied as seedling growth phase is the best to analyze such effects. The aqueous irradiated solution of oligosaccharide was applied in the following 2 modes, namely, presoaking of the seeds with different concentrations of irradiated and non-irradiated CS with water as control before sowing and different concentrations of CS solution both irradiated and non-irradiated with a control used for irrigation after sowing. The results of the study showed that, non-irradiated and irradiated CS did not show any effect on the germination. The percentage of germination and the emergence of

radical were not affected at all. All samples of irradiated CS promoted the seedling growth the root growth and proliferation in both crop species. The maximum height was obtained using CS samples irradiated to 15 kGy. Further increase in dose did not affect the biological activity of oligosaccharides. All concentrations ranging from 20 ppm to 100 ppm were nearly equally effective in stimulating the seedling growth. The concentration of oligosaccharide in excess of 100 ppm upto 500 ppm did not prove any additional beneficiary or adverse effect. These results clearly showed that irradiated CS was effective in promoting plant growth.[36] have successfully demonstrated the application of these CS oligomers in three different crops namely, Triticum aestivum (Wheat), Vigna radiate (Beans) and Linum usitatissimum (Linseed) as growth promoter. Used different treatments included Dry seed treatment; Water presoaking treatment; Irrigation treatment and Foliar spray treatment. To determine the effective dose of gamma radiation the dry seeds of wheat, beans and linseed were treated with 500 ppm of oligo-CS, produced by different doses of gamma radiation. This experiment was performed specifically to determine the effective dose of gamma radiation for the production of oligo-CS of desired molecular weight with maximum biological activity. In this investigation gamma radiation doses Viz., 15, 35, 65, 77 and 89 kGy were used for the production of oligo CS. All the doses of gamma radiation were found to be effective in degrading the molecules of CS. The most effective dose of gamma radiation was determined on the basis of stimulation in seedling growth, induced by oligo-CS. The lowest dose of 20 kGy of gamma radiation was marginally effective. However, the increase in the dose of gamma radiation to 35 and 65 kGy increased the growth stimulating activity of both compositions. Further increase in the dose of gamma radiation up to 89 kGy led to decline in the stimulatory effect of CS. However, at the higher doses of gamma radiation, CS still retained the stimulatory effect on the seedling height. In the water control, the seedling height in wheat, mung bean and Linum was 12.61, 9.50 and 8.40 cm, respectively. The unirradiated CS did not show any stimulatory effect on the seedling growth in all the three species [37]. Utilized radiation processed CS as plant growth promoter carrying out the experiment in the 24 hectares of rice crops. For the field trial, a pilot scale production of oligo-CS was established using gamma irradiation for partial degradation of CS powder followed by gamma irradiation of aqueous solution of 3% irradiated CS powder in 2% lactic acids (3CL2). Radiation dose of 50 kGy was selected for initial degradation of CS powder and followed by 12 kGy irradiation of 3CL2 dissolved CS. On an average oligo-CS with molecular weight ~10,000 was obtained and subsequently used in the field trial on MR219 type of rice seeds planted in 24 hectares of rice plots. The seedlings were carried out after the rice seeds were soaked 24hrs in water and 30 minutes in 200 ppm oligo-CS. The rice plots that were sprayed with oligo-CS were found to have higher resistant towards blast diseases. Oligo-CS of 40 ppm was found to be effective as fungicides and resulted in the increase of yield of rice seeds of about 5%. Effects of CS on the morphological properties on the plants/fruits were investigated by Hassan et.el. (2009) [38]. By spraying the irradiated and non-irradiated CS solutions on various vegetables namely, Ammaranthus Cruentus (Local name: Datasakh) and green chili plants. Their

results showed that irradiated CS solution is effective as plant growth enhancer. CS enhances the vegetative growth in terms of the average values of stem length, number of growing leaves, including leaf width and length etc. Controlling degradation process of CS by gamma radiation from a Co^{60} source was investigated by El-Sawy et. al. (2010) [39]. Controlling was done using different doses in powder form and in presence of additives. The efficiency of these methods was verified by viscometric and GPC analysis and the average molecular weight of degraded CS were determined. The irradiation degradation in the presence of chemical initiator was much more appropriate from economical point of view because it reduced the irradiation doses required for degradation. Characterization of degraded polymer by FT-IR spectroscopy, UV–visible spectroscopy, XRD, ESR and TGA analysis was investigated. The degraded natural polymer CSs were tested as growth promoters for Zea maize and bean plant and degraded CS was found to have a great effect on the productivity and properties of these plants. Controlling the degradation of CS by gamma radiation from a Co^{60} source in presence of initiators such as ammonium per-sulfate and hydrogen peroxide, and subsequent use of the degraded CS as plant growth promoter was performed by Thama et. al. (2001)[40]. The factors affecting the degradation process such as irradiation dose, type of initiator and its concentrations were studied. The water-soluble CS separated from degraded CS prepared at different irradiation doses showed a strong effect on the growth of Faba bean plant and can be used in agriculture fields as a growth promoter [41]. The toxicity of vanadium (V) and the effect of CS on soybean, rice, wheat and barley. Wheat and barley were sensitive to V than rice and soybean but all seedlings of these plants were damaged at 2.5 μg/mL V (in VCl_3). These damages were reduced by application of radiation-degraded CS. The recovery of growth and reduction of V levels in seedlings were obtained by the treatments with 10–100 μg/mL CS irradiated at 70–200 kGy of γ-rays in 1% solution. The reductions of V and Fe contents in plants were due to the ability of CS to form chelate complexes with metals in solution. The results of Bangladesh agricultural society analysis showed that the absorption and transportation of 48 V to the leaf from root was suppressed with irradiated CS. Therefore, it can be concluded that CS irradiated at suitable doses (ca. 100 kGy) is effective as plant growth promoters as well as heavy metal eliminators in crop production.

1.10.3 Irradiated CS for food preservation

The preservation of food by physical and chemical methods is an important area to the food industry. When physical means of preservation are not available or desirable, chemical preservative must be used, and the choice of one or more preservative is primarily based on the chemical composition of the food, its pH, and other characteristics. Food preservatives have to meet several regulatory standards, for example, they must be (i) efficient over a broad range of spoilage organism; (ii) tasteless and odorless, (iii) non-toxic, (iv) safe and (v) inexpensive. No preservative is universal, and the list of GRAS (generally recognized as safe) preservatives is short[42,43].Presently for fruit storage many synthetic/soft chemicals viz., inorganic

copper compounds, colloidal sulphur, sulphur compounds, phenolic compounds, dithiocarbamates, antibiotics, calcium compounds and various fungicides have been used. Although the relevance of chemicals as preservatives in today's perspective is not positive regarding environment and consumers health. Fruit sensitivity to decay and general fruit perishability due to the rapid ripening and softening limits the storage, handling and transport potential [44]. On the other hand, application of modified atmosphere (MA) or controlled atmosphere (CA) is not always compatible with this fruit. Although CA storage has been shown to extend the shelf-life of mango [45, 46], it is cost prohibitive. MA storage was also reported to slow mango ripening, but was often accompanied by high CO_2 and off flavor [47]. These problems in food preservation has been the center of concern for scientists creating a growing demand for natural preservatives, and for these reasons, alternative sources of safe, effective, and acceptable preservatives need to be developed. For this reason researcher do research on natural occurring preservation like CS. The structure of CS resembles with the cellulose except that the hydroxyl groups in position 2 have been replaced by acetyl amino groups [48]. CS is a very reactive polysaccharide having three different functional groups (primary −OH, secondary –OH and $-NH_2$) and water soluble with organic acids [49]**.** It inhibits the growth of a wide variety of bacteria [50] and fungi [51-55] have ascribed the function of high-molecular-weight CS as an antimicrobial material or flocculent to either amino groups in the molecule or hydrogen bonding between CS and extra cellular polymers in addition to an electrostatic interaction with the cell surface[56]. The combined effect of CS and cellulosic material on the flocculation, the antimicrobial activity of CS degraded by chitinase and showed that the activity of 5% degraded CS was higher than that of un-degraded one. Furthermore, the use of degraded CS as food preservative was also investigated [56, 57] achieved a 10 day shelf life extension of "mulkimchi" 9 (pickle type kimchi, i.e., Chinese cabbage) at 5°C by incorporating 0.2% CS compared to control samples. CS can be used as edible coating, which is defined as "a thin application of material that forms a protective barrier around an edible commodity and can be consumed along with the coated product" [58]. Edible coatings are used to create a modified atmosphere, to improve appearance and to reduce weight loss during transport and storage [59]. The barrier characteristics to gas exchange for films and coatings are the subjects of much recent interest. Development of films with selective permeability characteristics, especially to O_2, CO_2 and ethylene allow some control of fruit respiration and can reduce growth of microorganisms. Coatings have long been used on citrus, apples (shellac and carnauba wax), tomatoes (mineral oil) and cucumbers (various waxes). However, these coatings are less studied for use on apricot [59], pineapples, bananas, cherries, dates, guavas, mangoes, melons and nectarines or peaches [60]. Nevertheless, the postharvest use of polysaccharide and protein coating materials on several types of fruit has been developed in the past few years including cellulose-sucrose fatty acid esters on apricot [59], cellulose on mango, guava[60], CS on strawberry [61], tomato [62] and the corn protein (Zien) on tomato [63]. CS is well known coating material used in several fruits for prolonging their shelf life [64,65]. Similarly, irradiation is an economically viable technology for reducing postharvest losses and

maintaining hygienic quality of fresh produce [66, 67]. Mango being a highly perishable fruit possesses a very short shelf life and reach to respiration peak of ripening process on 3rd or 4th day after harvesting at ambient temperature.The shelf life of mango varies among its varieties depending on storage conditions. It ranges from 4 to 8 days at room temperature and 2-3 weeks in cold storage at 13ºC. This short period seriously limits the long distance commercial transport of this fruit [68]. Usually after harvesting, the ripening process in mature green mango takes 9-12 days [69]. The ripening process of mango fruit involves a series of biochemical reactions, resulting into increased respiration, ethylene production, change in structural polysaccharides causing softening, degradation of chlorophyll, developing pigments by carotenoids biosynthesis, change in carbohydrates or starch conversion into sugars, organic acids, lipids, phenolics and volatile compounds, thus leading to ripening of fruit with softening of texture to acceptable quality [70]. Abbasi et al. (2009) [71] evaluated different types of irradiated CS coatings most suitable for enhancing the shelf life and improving quality of mango fruits. Effect of coating with irradiated Crab and Shrimp CS (CHIirr, Mv = 5.14 × 104) and un-irradiated Crab CS (CHIun, Mv = 2.61 × 105) on postharvest preservation of mango (Mangifera indicaL.) fruit have been studied. The effect of various CS coatings on fruit ripening behaviour, biochemical and organoleptic characteristics were evaluated during storage. The incidence of disease attack was also observed. CS treated fruit inhibited the growth of a wide variety of bacteria and fungi as compared to the control treatments. The fruit-spoiling fungi (Colletotrichum gleosporioides) were observed in untreated control fruits after 2 weeks and in irradiated CS coated fruits after 5 weeks of storage. The control fruits were affected 13.3%, after 14 days of storage while irradiated CS coated fruits were affected only 6.9%. At the end of storage control fruits were fully spoiled. However, irradiated CS coated fruits were still having 75% fruits not having disease attack. El-Ghaouth et al., (1991) [60] suggested that CS induces chitinase, a defense enzyme which catalyzes the hydrolysis of chitin, a common component of fungal cell walls, thus preventing the growth of fungi on the fruit. The results suggest that irradiated CS coating is effective on preservation of fresh fruits and it can extend the shelf life [78], limit the growth of fungi, and decrease the spoilage without affecting on ripening characteristics of fruit. Microbial inhibition mechanism of original CS abides mainly by only one mechanism in which CS molecule stack to cell wall [72]. It has been proposed that when CS is liberated from the cell wall of fungal pathogens by plant host hydrolytic enzymes CS penetrates the nuclei of fungus and increases with RNA and protein synthesis [73-74]. CS coated tomatoes were prevented by attack of Penicillium spp., Aspergillus spp., Rhizopus stolonifer and Botrytis cinerea. Moreover, CS has itself ability to control some fungal diseases, which deteriorate fruit quality during storage studied. Some experiments were also done on Papaya (Carica papaya L.) which is a very common tropical fruit. During post-harvest stage almost 20-30 % of papaya is lost. Post-harvest deterioration of papaya is a microbiological process; the fruit becomes target of several pathogens in the market thus decreasing its acceptability and shelf life. The storage life of papaya was 11 extended upto 33 through use of calcium chloride (2 %) with CS coatings on fruits. CS coating

impregnated with fungicides was also reported to prevent post-harvest loss of papaya fruits [75] to a greater extent compared to fungicides only.

1.10.4 Irradiated CS as effective fungicide

The enhancement of antimicrobial activity of irradiated CS was investigated by Matsuhashi and Kume (1997) [76]. It was concluded that irradiated CS having molecular weight of 105 to 3 × 105 exhibited highly antimicrobial activities. Ha et al. (1999) [77] recognized the enhancement of antifungal activity of irradiated CS for different fungi strains. Many researchers reported that the preparation of oligo-CS by radiation degradation of CS in solid state and in solution. Results indicated that in order to obtain oligo-CS in case of irradiation of CS solution, the dose should be higher than 10 kGy while very high dose and followed by fractionation should be needed for irradiating CS in solid state. The irradiation effect on CS in solid state to regulate CS molecular weight, the yields of oligo-CS by irradiating CS solution and biological activities of resultant products such as anti-fungi, pisatin induction and plant growth-promotion. CS degraded by irradiation with radiation degradation yield of about 1.03 (scissions/100 eV) in solid state. Oligo-CS DP < 8 with 50 % mass fraction can be obtained by irradiating 10% (w/v) CS solution with gamma Co-60 radiation at dose of 45 kGy for CS having initial Mv = 60,000. Hanh et al. (2004) [78] studied degradation of CS by combination of oxidation reagent and irradiation with Co-60. Resultant products were tested in the field for prevention of infection by Rhizoctonia solani that is one of the serious pathogenesis fungi for rice plants in the tropics. The antifungal effect of irradiated CS at dose of 50 kGy and concentration of 80 ppm was most effective. CS was also proven effective against gray mold disease caused by fungus Botrytis cinerea is considered an important pathogen around the world. It induces decay on a large number of economically important fruit and vegetables during the growing season and during post-harvest storage. It is also a major problem to long distance transport and storage. This fungal pathogen infects leaves, stems, flowers and fruit, either by direct penetration or through wounds caused by cultivation practices. The Control of this disease is especially important during storage because it develops at low temperatures (− 0.5 °C) and spreads quickly among fruit and vegetables. The degraded CS enhances fungal decay when used at high concentration dose. The effects of CS oligomers on gray mould caused by Botrytis cinerea in tomato plants were investigated by Hassan et al. (2009) [79]. It was found that degraded CS controlled the gray mould disease caused by B. cinerea compared with control. Oligo-CS has been recognized as potent phyto-alexin inducer (elicitor) to resist infection of diseases for plants [80-82]. Thus it is promising to utilize oligosaccharides particularly oligo-CS in agriculture as biotic elicitor to enhance defense responses against diseases and as growth promoter for plants [82-84]. Several authors also proved the antifungal effect of degraded CS. Results showed that CS having degree of polymerization (DP) from 2 to 8 has been recognized as phytoalexin inducer to prevent infection of Pyricularia Oryzae in suspension cultured rice cells [85]. Roby et al. (1996) [85] demonstrated oligomers from hexamer to nonamer of oligo-CS

induced elicitor activity for infection inhibition by Colletotrichum lagenarium in melon plant. Thus, oligo-CS is very promising to utilize in agriculture for enhancement of immune system against infection of diseases in plants. Hien (2009) [86] prepared CS from shrimp shell (alpha CS) and from squid pen (beta CS) with degree of deacetylation of about 70%. Degradation of CS in flake form by combined treatment with H_2O_2 and gamma Co-60 radiation was carried out. Results showed that combined treatment was highly effective for degradation of CS to obtain low molecular weight of 1-2×105. Oligo-CS was prepared by irradiation of CS solution of 50 g/L (5%, w/v). The dose required for oligo-CS with water soluble content of more than 70% was of 32 kGy and 48 kGy for beta and alpha CS, respectively. Synergic effect of degradation of CS in solution with H_2O_2 and gamma Co^{60} radiation was also investigated. The dose to obtain oligo-CS was reduced from 32 kGy to 4 kGy for beta CS and from 48 kGy to 8 kGy for alpha CS. The elicitation and growth promotion effect of oligo-CS for sugarcane and rice were investigated. Results showed that oligo-CS with water soluble content of 70-80 % (Mw~5,000-10,000) exhibited the most effectiveelicitation and growth promotion for plant. The optimum oligo-CS concentration by spraying was of 30 and 15ppm for sugarcane and rice, respectively. The disease index of Ustilgo scitaminea and Collectotrichum falcatumon sugarcane were reduced to 44.5 and 72.3% compared to control (100%). The productivity of sugarcane was increased about 13% (8tons/ha). The disease index of Pyricularia griseaon rice was reduced to 53% for leaf and 34% for neck of bloom compared to control (100%). The productivity of rice was increased for 11-26 % (0.6-1.4 tons/ha). The obtained results indicated that oligo-CS is promising to use as a biotic elicitor for plant particularly for sugarcane and rice. Fidelis et al. (2009) [87] used CS, extracted from sea crab shells to determine antifungal properties against Aspergillus niger. CS powder irradiated at 100 kGy and dissolved in 1% acetic acid (v/v) with p^H adjusted to approximately 6.0 was used in preparing CS concentrations of 2 %, 1.5 %, 1 % and 0.5 %. The agar dilution method was used to test the antifungal activity of the various CS solutions at concentrations of 0.20 %, 0.15 %, 0.10 % and 0.05 %. Both media containing irradiated and unirradiated CS inhibited the mycelial growth of Aspergillus Niger and the degree of inhibition was dependent on the concentration of the CS in the fungal growth medium. Results showed that the media containing irradiated CS inhibited the mycelia growth of Aspergillus Niger to a greater extent than the media containing unirradiated CS.

1.11 Piper Betel

The Piper betel [88] is the leaf of a vine belonging to the Piperaceae family, which includes pepper and kava. It is valued both as a mild stimulant and for its medicinal properties. Betel leaf is mostly consumed in Asia and elsewhere in the world by some Asian emigrants, as betel quid or in paan. The betel plant is an evergreen and perennial creeper, with glossy heart-shaped leaves and white catkin. The betel plant originated from South and South East Asia. Betel or bétele (Portuguese) derived from the Malayalam word vettila. Vetel in Malayalam means Betel and ila means leaf.

1.11.1 Cultivation

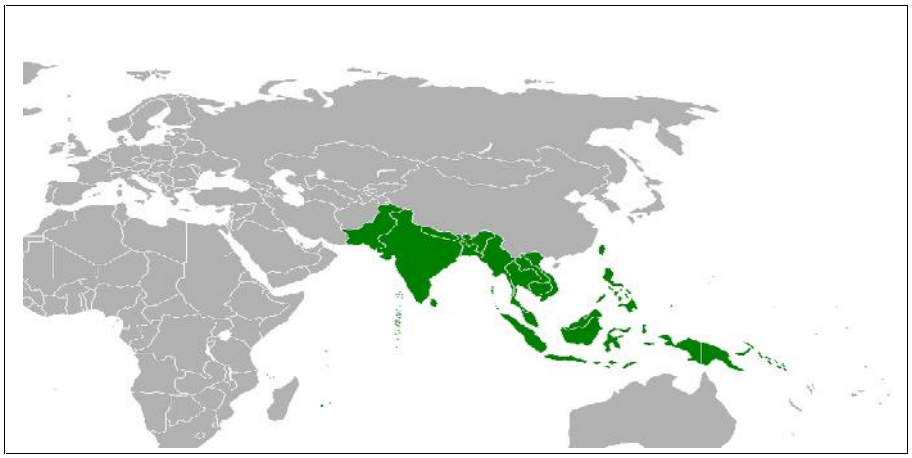

Figure 18: Betel Plant cultivation in South Asia [89].

The betel leaf is cultivated mostly in South and Southeast Asia, from Pakistan to Papua New Guinea. Since it is a climbing plant, it needs a compatible tree or a long pole for support. Betel requires well-drained fertile soil. Water logged, saline and alkali soils are unsuitable for its cultivation. In Bangladesh, farmers called barui prepare a garden called a barouj in which to grow betel. The barouj is fenced with bamboo sticks and coconut leaves. The soil is plowed into furrows of 10 to 15 metres' length, 75 centimetres in width and 75 centimetres' depth. Oil cakes, manure, and leaves are thoroughly incorporated with the topsoil of the furrows and wood ash. The creeper cuttings are planted at the beginning of the monsoon season.

Figure 19: Photograph [90] of betel vine cultivation in Coxsbazar, Bangladesh.

Proper shade and irrigation are essential for the successful cultivation of this crop. Betel needs constantly moist soil, but there should not be excessive moisture. Irrigation is frequent and light and standing water should not remain for more than half an hour.

Dried leaves and wood ash are applied to the furrows at fortnightly intervals and cow dung slurry is sprinkled. Application of different kinds of leaves at monthly intervals

is believed advantageous for the growth of the betel. In 3 to 6 months the vines reach 150 to 180 centimeters in height and they will branch. Harvest begins, with the farmer plucking the leaf and its petiole with his right thumb. The harvest lasts 15 days to one month. Betel plant has made its way to research labs of many Bangladesh chemical and food nutrition companies.

The harvested leaves are consumed locally and exported to other parts of Asia, the Middle East, Europe, and the Americas. Betel is grown and cultivated as an important crop in rural Bangladesh. Last year, India exported betel to 28 countries. Pakistan is the biggest importer of Indian betel.

1.11.2 Diseases in betel vine

The perennial crop is found to be infected by various diseases [91] of which leaf rot and leaf spot caused by pathogens, Phytophthora parasitic and Colletotrichum capsici are the major constraints for cultivation of the crop across the country. Leaf rot can damage the crop within a week when it attacks the vine. Leaf rot and foot rot have been reported [92] to be caused by Phytophthpra palmivora and leaf rot may cause 30-100% leaf yield loss. The relative humidity enhances the incidence of the leaf rot disease Leaf rot disease of betel leaf (Pan) was influenced by high atmospheric humidity and rainfall from June to August annually. On the other hand, incidence of leaf spot increased with less humidity gradient during the months of November to March. In November 2008, betel vines (Piper betel L., Piperaceae) exhibiting leaf blight symptoms were observed in central, Taiwan. Infections resulted in a 30 to 70% loss of leaf yield in the investigated betel leaf-producing facilities. Symptoms began with small, necrotic, water-soaked spots that progressed to circular to irregularly shaped, with chlorites halos on leaves; some lesions started from the edge of leaves and later fused to form dried, necrotic margins. Five bacterial isolates from three lesions were characterized with fatty acid methyl ester analysis and for each isolate, the bacterium was confirmed as Acidovoraxavenuesubsp. citrulli with a similarity index >0.70. In addition, the Biology system (Biology, Hayward, CA) and 16S ribosomal RNA sequence identity comparison were performed to confirm that the five betel vine-isolated bacteria were A. avenuesubsp. citrulli based on a similarity of 0.54 with biology and 99% sequence identity for 16Sr RNA gene. Koch's postulates were fulfilled by infiltrating a bacterial suspension of 3 × 105 CFU/ml into 40 leaves of four greenhouse-grown, disease free, mature betel vine plants. After inoculation, plants were kept in a humidified greenhouse at 28°C to favor symptom development and symptoms similar to those observed in the greenhouse were evident at 7 days post inoculation (dpi) onall bacterium-infiltrated leaves. Control leaves infiltratedwith distilled water remained symptomless. Bacteria showing morphological and biochemical similarities to the ones used for inoculation were isolated from all of the inoculated betel vine leaves. In addition, a bacterial suspension at 3 × 108 CFU/ml was sprayed at the amount of 5 ml per plant onto 6 to 10 plants each of 4-week-old disease-free seedlings of watermelon (Catulluslunates Matson & Nakai, cv. Empire

no.2), oriental sweet melon (Cucumis melo L. var. saccharin's Naudin, cv. Silver Beam), and wax gourd (Beniciahispid Cogn.) for bio assays, and the inoculated seedlings were enclosed in plastic bags for 36 h at 28°C. Water-soaked lesions were observed on leaves of watermelon and wax gourd at 2 dpi and on sweet melon at 4 dpi on all inoculated plants but not on distilled water-sprayed control plants, indicating that A. avenue subsp. citrulli strains from betel vine could also infect melon plants. Betel vine plants are infected variety of diseases in the entire plantation without any early indications of the diseases. Betel vine plants are infected variety of diseases in the complete plantation without any premature warning of the diseases.

1.11.3 Current economic situation

Betel leaf (Paan) exported to the European and Middle Eastern countries stood at over US $ 31 million in 2012, according to government figures. Detection of Salmonella bacteria in betel leaf from Bangladesh in the UK prompted the European Union tosuspend imports. Saudi Arabia and the USA are other big markets for betel leaf.

Figure 20: Betel Plant selling from garden to market in Cox's bazaar Bangladesh.

The government has taken an initiative to produce bacteria-free betel leaf in order to resume its export to the European countries which had previously banned betel leaf from Bangladesh following the detection of a virus Salmonella, which is injurious to health. We are hopeful of making our betel leaves bacteria-free to resume its export by this research.

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1.13 Aims and Objectives

- To develop a natural polymer based plant growth promoter to reduce the use of chemical fertilizer.
- To prepare sodium alginate, chitosan and their mixtures.
- Use them after irradiation as plant growth promoter on selective crops (tomato, cabbage, cauliflower, spinach, mungdal, pumpkin, jute, eggplant, green chilli, red chilli plants and betel vine).
- To analyze growth of piper betel vine with respect to their heights, size of the plants, number of leaves, leaves areas, number of stems, length of their roots, fruits size, thicknesses of the vine and weights.
- To analyze nutrient and heavy metal contents in the betel leaves after the application of the sodium alginate and chitosan.
- To analyze heavy metal contents in the soil of cultivation site of the experimental betel vine garden and out of the garden.
- To observe disease control action on selective crops after the application of sodium alginate and chitosan.
- To observe disease control action, antifungal nature and shelf life effect on betel leaves after the application of sodium alginate and chitosan.
- To conduct sensory evaluation test of betel leaves after the application of sodium alginate and chitosan and and their mixtures on betel vines.

Chapter 2: Materials & Methods

2. Materials and Methods

2.1 Preparation of SA solution

SA, of high molecular weight was supplied from UNI-CHEM, China. 3% (w/v) solutions of SA was prepared from SA powder in distilled water and then this solution was irradiated to Co^{60} γ-irradiation at 12 kGy at dose rate of 5 kGy/h. Irradiated solution were then diluted to various concentrations (100 ppm to1000 ppm). Nonnirradiated SA and various concentrations of irradiated SA were used for further investigation.

2.2 Preparation of CS in conventional method

The locally collected waste prawn shell was washed with hot water and dried in an oven at 105°C for 72 hours (scheme 1).

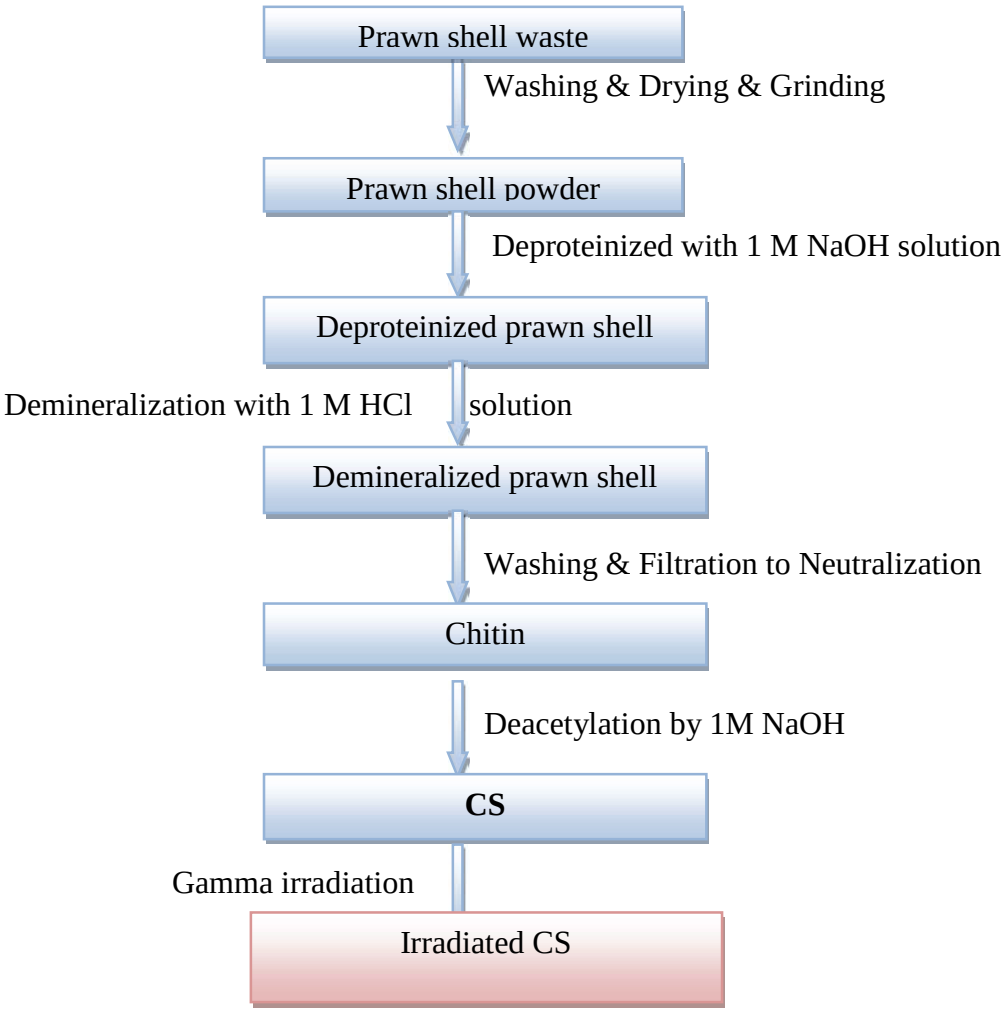

Figure 1: Scheme for the process of extraction CS from irradiated chitin samples.

Dried and ground prawn shell was then deproteinized with 1 M NaOH solution at around 100°C temperature for 4 hours in a ratio of prawn shell and NaOH at 1:16 on a w/v basis and demineralized with 1 M HCl solution at same temperature for 4 hours at a ratio of chitin at HCl solution at 1:13 on a w/v basis. The mixture was then washed with distilled water until completely filtered to neutralize and dried at 105°C in an oven for 24 hours. Prepared chitin is an intermediate product of CS. CS was obtained by deacetylation of chitin using NaOH again at a ratio of chitin and 1 M NaOH solution at 1:20 (w/v) at 100°C for 3·5 hours. Then prepared CS was filtered from the solution of alkali and was extensively washed with distilled water to remove traces of alkali. The resultant CS was dried in a vacuum oven at 50°C for 24 hours. 2% (w/v) aqueous solutions of prepared CS were irradiated at 2-100 kGy for further use.

2.3 Co^{60}gamma source

Gamma sources are generally characterized as electromagnetic radiation having the highest frequency and energy, and also the smallest wavelength (below about 10 picometers) within the electromagnetic spectrum. The 3% SA and 2% CS solution were irradiated by gamma rays from Co^{60} source (Strength: 25Kci, Type T-T 252 loaded with Co^{60} pellets, This unit has 12 tubes, each containing three active capsules interspaced with springs). Model Gamma beam is loaded with source GBS-98 that comprises of thirty six encapsulated capsules in the "Institute of Food and Radiation Biology (IFRD)" at Bangladesh Atomic Energy Research Establishment, Savar).This Co^{60} Gamma source is used for this experiment. Accuracy of dose rate is ±1% at 99% confidence.

2.4 Determination of viscosity average molecular weight of polymer

Viscosity is an internal property of a fluid that offers resistance to flow. It is due to the internal friction of molecules and mainly depends on the nature & temperature of the liquid. Many methods are available for measuring viscosity of polymer solution. The Ostwald method is a simple method for the measurement of viscosity, in which viscosity of liquid is measured by comparing the viscosity of an unknown liquid with that of liquid whose viscosity is known. In this method viscosity of liquid is measured by comparing the flow times of two liquids of equal volumes using same viscometer. Consider two liquids are passing through a capillary of same viscometer. Then the coefficient of viscosity of liquid (η_2) is given by equation:

$$\eta_2 = \frac{\eta_1 \rho_2 t_2}{\rho_1 t_1} \qquad \text{----------(Equation 1)}$$

Heret_1 andt_2 are the time of flow of the liquids and$\rho_1$and $\rho_2$are the respective densities. And η_1 is the coefficient of viscosity of water. For a given liquid η has a specific value at the same temperature. Various mixtures of two non-interacting liquids viscosities will lie among the viscosities of those pure components. The time of flow

of liquid depends on the viscosity and composition. In this method the flow times are measured for different known compositions and a graph is plot for time of flow and compositions. The unknown composition can be determined by plotting a graph for the time of flow and compositions. The molecular weight of the polymer is measured by using viscometer and the molecular weight obtained by this technique is called viscosity average molecular weight. The molecular weight of the polymer solution is very high so the viscosity of polymer solution is very high compared to that of pure solvent. From the Mark-Houwink equation the relationship among the molecular weight and viscosity are given below-

$[\eta]=KM^a$ ---------- (Equation 2)

Where [ŋ] is the intrinsic viscosity, [M] is Molecular weight, [K] and [a] are constants for a particular polymer solvent system. If we know the [K] and [a] values for a given polymer solution the intrinsic viscosity and molecular weight can be calculate using the above equation 2.

Terms related to viscosity measurements by the following equations-

Relative Viscosity $$\frac{\eta}{\eta_0}=\frac{t}{t_0}=\eta_r$$ ---------- (Equation 3)

Specific Viscosity $$\frac{\eta-\eta_0}{\eta_0}=\frac{t-t_0}{t_0}=\eta_r-1=\eta_{sp}$$ ---------- (Equation 4)

Reduced Viscosity $$\frac{\eta_{sp}}{C}=\eta_{red}$$ ---------- (Equation 5)

Inherent Viscosity $$\frac{\ln \eta_r}{C}=\eta_{inh}$$ ----------(Equation 6)

Intrinsic Viscosity $$\left(\frac{\eta_{sp}}{C}\right)_{C\to 0}=\left(\frac{\ln \eta_r}{C}\right)_{C\to 0}=[\eta]$$ ----------(Equation 7)

For measuring intrinsic viscosity of polymer sample, solutions of known concentrations are prepared, the flow times of solvent (t_0) and the solutions (t) are measured using viscometer.

Double extrapolation plots of reduced viscosity against concentration and inherent viscosity against concentration is plotted by calculating the corresponding reduced viscosity and inherent viscosity. The intrinsic viscosity is given by the common ordinate intercept of the figure 2.

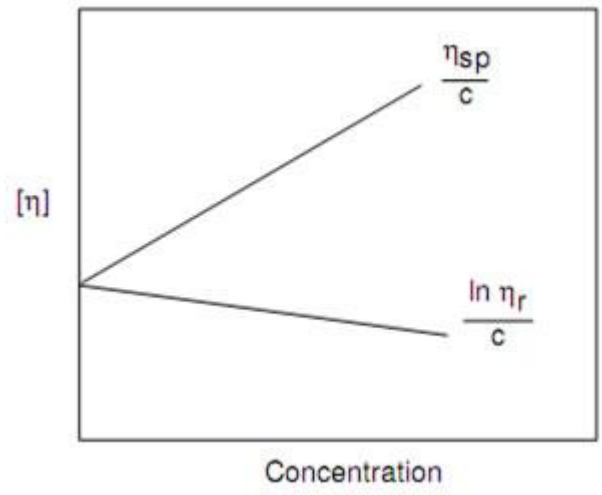

Figure 2: Intrinsic viscosity against concentration (g/ml) [3]

2.4.1 Viscosity average molecular weights (M_v) of the SA and CS

Viscosity average molecular weights (M_v) of the SA and CS were determined with an Ostwald Viscometer. For the measurement of viscosity average molecular weight of SA. SA powder was dissolved in 0.1M NaCl solution. For the determination of viscosity-average molecular weight of CS, the CS was dissolved in a mixture of 0.1M acetic acid (different concentration of 0.5%, 1%, 3%, 5%, 7% were prepared and 1% solution of them were prepared with 0.2 M NaCl and then the viscometer was used to measure the relative viscosity. Plotting the reducing viscosity ($\eta_{reduced}$) along Y-axis should give a straight line and when extrapolated to concentration=0, gives a value for [ŋ], called intrinsic viscosity.

The Mark Houwink equation [ŋ] =KM^a, relating to intrinsic viscosity with empirical viscometric constant K=1.81×10^{-}3 ml/g and a= 0.93 for CS was used to calculate the molecular weight. The molecular weight of SA can be determined by measuring the intrinsic viscosity of polymer in 0.1 M NaCl solution taking K = 8.1 × 10^{-}3 ml/g and α = 0.92revealed by Lui et al., 2002[1]. Using the above equation and from the intrinsic viscosity, the molecular weight was estimated. The viscosity average molecular weight was determined by equation 2.

2.5 Fourier transforms infrared (FTIR) spectroscopy

The FTIR spectra of irradiated and nonirrdiated SA and CS were analyzed by using a FTIR Spectrometer (Model: 01831, SHIMADZU Corp. Japan). For this purpose pellets of CS and SA (SA) were made with solid KBr separately. Approximately 2 mg of CS powder and 200mg of KBr was blended and triturated with agate motor. The mixture was compacted using an IR hydraulic press at a pressure of 60 tons for 60s. The samples were placed in the holder directly in the IR laser beam. The spectra were recorded in both cases in the transmittance band mode in the range of 4000-400 cm^{-1}.

2.6 Determination of degree of deacetylation (DD) of prepared CS samples

The Degree of Deacetylation of chitin varies with the change of 1) Heating time and 2) The concentration of NaOH solution.The degree of deacetylation (DD) of the CS was calculated using equation (8) from Baxter et.al. (2003)[2]with the help of IR spectrum. The computation equations for the baseline is given below-

$DD= 100- [(A_{1655}/ A_{3450}) X 115]$ ———— (Equation 8)

Where A_{1655} and A_{3450} were the absorbance at 1655 cm^{-1} of the $amide^{-1}$ band as a measure of the N-acetyl group content and 3450 cm^{-1} of the hydroxyl band present in CS. The baseline proposed by Baxter et.al. (2003) was modified from the reported by Domszy and Roberts were the computation equation is

$DD= 100- [(A_{1655}/ A_{3450}) X 1.33]$ ———— (Equation 9)

The 1.33 denoted the value of the ratio of A_{1655}/ A_{3450} for fullyN-acetylated CS. It was assumed that the value of this ratio was zero for fully deacetylated CS and there was a rectilinear relationship between the N –acetyl group content and the absorbance of the amide-1 band.

2.7 Scanning electron microscopy (SEM) of SA, CS and SACS samples

A SEM images of a sample by scanning it with a high-energy beam of electrons. The electrons interact with the atoms that make up the sample producing the signals that contain information about the sample's surface topography, composition and electrical conductivity etc. samples were put into a carbon tape on the sample holder of the SEM machine [JSM-6490 LA (JEOL) Ltd., Japan] at 20kV. The magnification (5-20000 µm) was controlled to a different resolution so that the structures of the SA, CS and SACS treated betel leaves were fully visualized.

2.8 Thermogravimetric analysis (TGA) of SA, CS and SACS samples

Effect of irradiation on the thermal properties of the irradiated, NCS, SA and SACS treated betel leaveswere observed by TGA measurements.Thermogravimetric analysis (TGA) was done by TGA-50 (Shimadzu, Japan) at a heating rate of 10°C/min from 30 to 600°C using aluminum cell under nitrogen atmosphere.

2.9 Flame Atomic Absorption Spectrometry (FAAS)

Atomic-absorption (AA) spectroscopy uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids or solids, the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined from the amount of absorption.

Applying the Beer-Lambert law directly in AA spectroscopy is difficult due to variations in the atomization efficiency from the sample matrix, and nonuniformity of concentration and path length of analyte atoms (in graphite furnace AA). Concentration measurements are usually determined from a working curve after calibrating the instrument with standards of known concentration.

Beer-Lambert Law is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:

$$A = a(\lambda) * b * c$$

Where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length, and c is the analyte concentration. When working in concentration units of molarity, the *Beer-Lambert law* is written as:

$$A = \varepsilon * b * c$$

Where ε is the wavelength-dependent molar absorptivity coefficient with units of

M^{-1} cm^{-1}. Data are frequently reported in percent transmission ($I/I_0 * 100$) or in absorbannce [$A = \log (I/I_0)$]. Experimental measurements are usually made in terms of transmittance (T), which is defined as:

$$T = I / I_o$$

Where I is thelight intensity after it passes through the sample and I_o is the initial light intensity. The relation between A and T is:

$$A = -\log T = - \log (I / I_o).$$

2.10 Preparation of SA, CS, SACS mixture

- **Preparation of SA:** 500 ml of 3% SA aqueous solution was prepared and then treated with Co^{60} gamma rays at 12KGy and then this solution was diluted to 100 ppm, 200 ppm, 300 ppm, 500 ppm, 1000 ppm and applied on the field trial spraying at 15 days intervals on Tomato, Spinach, Mung dal, Jute, Pumpkin, Egg plant, Green chilli, Red chilli, Cabbage, Cauli flower, upto 30 to 90 days.
- **Preparation of CS:** 500 ml of 2% CS solution was prepared and then treated with Co^{60} gamma rays at 50 KGy and then this solution was diluted to 100 ppm, 200 ppm, 300 ppm, 500 ppm, 1000 ppm and applied on the field trial spraying at 15 days intervals on Tomato, Spinach, Mung dal, Jute, Pumpkin, Egg plant, Green chilli, Red chilli, Cabbage, Cauli flower, upto 30 to 90 days.
- **Preparation of SACS mixture:** 3% SA solution and 2% CS solution were prepared and then treated with Co^{60} gamma rays and then diluted to 100 ppm, 200 ppm, 300 ppm, 500 ppm, 1000 ppm. For comparative study, a mixture of 3% SA and 2% CS

(SACS) solutions were prepared at a ratio of (50:50)%, (70:30)%, (90:10)% and applied on the field trial spraying at 15 days intervals on betel vine plants for 90 days.

2.10.1 Foliar application of SA, CS and SACS on selective crops

The solutions (SA, CS and SACS) were separately used on through foliar spraying on selective crops at 15 days intervals upto 30 to 90 days and for Betel vines it applied at 15 days interval for 90 days. The entire research works were held in two districts. Betel leaves were treated in Coxsbazar and other vegetables were treated in Dhaka (Table-1 & 2).

2.11 Data collection and statistical analysis

Visualobservationsweremadeeverydayanddatacollectionsof plants are described in Table1.Threereplicateswerecarriedout in each experiment. All data were analyzed by SPSS software, version 15 using one-way ANOVA analysis. The level of statistical significance was set at 5% (p 0.05). All photographs are representative for the growth condition as described.

Experimental plot areas were separated for each solution. These plots were prepared by adding natural fertilizer (cow dung) in soil. The plots were watered when needed. A definite amount of SA, CS & their mixture SACS samples were treated on betel vine and selective plant specimens. Foliage treatments were made through a hand sprayer over the leaves and stems. Soil was treated was treated with the dispersed sample like regular water spraying. Visual observations were made every day and data were collected as required. Three replicates were carried out for each data collection.

- **Plant height:** The plant heights were measured from its base to the highest point. This was recorded in a chart with both the date and the height. It was repeated every 7 days.
- **Number of stem:** Number of stem was counted in every 7 days.
- **Size of fruits:** Size of fruits was measured by measuring tape after 90 days of experiments.
- **Number of fruits:** Number of fruits was counted in every 15 day.
- **Number of leaves:** The number of leaves of each plant was counted and recorded. Every visible leaf on the plant, including the tips of new leaves just beginning to immerge was also counted in every 7 days.
- **Surface area of leaves:** The leaveswas placed over a graph paper and traced as described in the literature of Mawgoud et al., 2010[3,15]. After that the square covered were counted to give an estimate of the surface area of each leaf on a plant and for each plant in the experiment was done. The amount of squares covered by the leaf was then converted to area by simple calculation. As the same types of graph paper

Table1: Field trial of SA, CS & SACS on selective crops

	Various solutions			**Parameter observed**
	SA	**CS**	**SA CS**	
Selective crops	Tomato Spinach Mung dal Jute Pumpkin Egg plant Green chilli Red chilli Cabbage Cauli flower	Tomato Spinach Mung dal Jute Pumpkin Egg plant Green chilli Red chilli Cabbage Cauli flower		*Plant height *Size of fruits *Number of fruits *Root length *Dry weight * Harvest time * Fast flowering
	Betel vine	Betel vine	Betel vine	*Plant height *No. of leaves *No. of stem *Leaves area *Leaves color *Vine's thickness *Root length *Wet & Dry weight *Reduction of diseases *Shelf life *Nutrient content *Heavy metal content *Soil test *Antifungal activity *Sensory evaluation * Self life

were used in every examination, the area was measured in a definite unit. As the smallest squire unit of the used graph paper has a length of a=0.3 cm, so the area of the smallest square is a^2= 0.9 cm^2. So a leaf on an area 50 smallest squares has the real area as 50=4.5 cm^2.

- **Leaves' color:** Any observation on changes on differences in leaves' color was done.
- **First flowering:** The number of days since initial planting to the first flower was recorded. For using flowering plants these two measurements serve as an additional indication of plant health.
- **Thickness of vine and root:** Thickness was measured by slide calipers on the first and last day of experiments.
- **Root lengths:** Root lengths were measured by slide calipers on the first and last day of experiments.

2.12 Measurement of the wet weight of the plants

The wet weight was measured by weighing the raw plants after plucked them from the plants and remove them from soil and washing them for removal of dust from leaves and soil from roots. The specimen plants (in some cases) were then cut into pieces to measure the weight of roots individually. The dried samples were then weighed and preserved.

2.13 Nutrient and Heavy metal determination in plants and soil

A number of macro and micro nutrients (e.g. Fe, Zn, Na, K, Ca), heavy metals (e.g. Cu, Ca, Ni, Co, Pb, As, Hg) consumption in betel leaves and in soil (of the experimental and outside of the experimental area of betel vine garden) were identified by Atomic absorption (FAAS) method in Chemistry division laboratory of Bangladesh Atomic Energy Commission, Dhaka.

2.14 Disease observation

Number of diseased leaves and plants were counted to estimate the tendency of disease after this experiment conducted.The effected leaves were counted in every 15 days.

2.15 Anti-fungal test of betel vine plant

The fresh betel leaves for different treatment were collected from plantation plots. 100 different samples were selected, which include 10 control, and 90 plant samples from plants treated with SA, CS and SACS. To prepare sample, 0.90% saline water was prepared from pure sodium chloride. Then 9 ml of saline water was poured into 20 ml size conical flask. Then 1 gm of betel leaves from each sample was soaked into corresponding conical flask followed by shaking to disperse fungus into saline water. Then 0.1ml saline water was inoculated onto PDA media. Then sample was spreaded using L-shaped spreader. After spreading, the inoculated PDA petri plate was incubated in incubator for overnight at 37^0C. The enumeration of fungal count was done the day after inoculation.

2.16 Shelf life observation

After 30 days spraying of irradiated SA, CS and SACS on betel vine plants, treated and control leaves were collected in air tight bag and kept in a normal refrigerator of IRPT laboratory of AERE.

2.17 The sensory evaluation test

Piper betel leaves treated by irradiated SA, CS, and SACS were collected after 30 days of foliar spraying. To conduct the sensory evaluation test**,** seunbiased serving procedure (i.e. indirect samples, interval between tasting, orders of presentation etc.) were followed. Rank positions (0-10) are taken for the average value of color, texture, appearance and taste. Differences between totals are tested for significance using Rank-Sum Difference tables.

2.18 References

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Chapter 3: Results and Discussion

(Section 3.1: Application of irradiated SA on selective crops)

3. Results & Discussion

Section 3.1: Application of irradiated SA on selective crops

3.1.1 Introduction

Low molecular weight naturally occurring polysaccharides like alginates, prepared by conventional methods, have been reported to possess novel features such as promotion of seed germination and shoot elongation of plants. As compared to the conventional techniques, like acid or base hydrolysis or enzymatic methods [1] radiation processing offers a clean one step method for the formation of low molecular weight polysaccharides in aqueous solutions even at high concentrations. Radiation can induce degradation of natural polymers like alginate and the degradation of solid SA requires high radiation doses. In fact, from the economic point of view, the cost of these doses is high. Therefore, trials have been made to reduce the cost of degradation process of solid alginates by adding chemical initiator to reduce the dose required for degradation and controlling the irradiation conditions. It was reported that the radiation-induced degradation yields of polysaccharides vary widely depending on the molecular weight of the polymer [2]. This is why; in this study the molecular weight of degraded fractions of alginate was determined. Viscometry is an essential tool to identify the molecular weight of alginates that were exposed to different irradiation doses.

3.1.2 Viscometric determination of molecular weight of irradiated of SA

Molecular weight of any polymer is one of the most key points which define its various properties in different applications. The conditions under which irradiation occurs can significantly influence the properties of the final materials. Alterations in the molecular structures of the polymers appear as changes in the chemical or physical properties. Alginate belongs structurally to polysaccharides for which is known that the irradiation conditions can significantly influence the properties of the final materials The molecular weight of raw alginate was reduced from 300000 to 25000 when irradiated at 100 kGy and the factors affecting the degradation process such as irradiation dose [3-4]. Effect of radiation on the molecular weight of SA was viscometrically monitored. The changes in the viscosity average molecular weights of SA due to radiation applied are shown in figure1. From the figure it is observed that as the irradiation dose increases the viscosity average molecular weight of SA decreases. Molecular weight have been found 9.87×10^{4} for NSA, whereas 3.14×10^{4}, 5.00×10^{3}, 3.34×10^{3}, 8.10×10^{2}, 5.60×10^{2}, 2.00×10^{2} were obtained for 5, 12, 20, 30, 40 and 50 kGy radiation dose applied[5-7]. Similar results were found by Hien et al.2000 [5] and Mollah et al.2009 [6]. They found that gamma radiation decreases the molecular weight by free radical depolymerization of SA [7]. At 12 kGy and 20 kGy irradiated samples showed significant decrease in molecular weight. The effects of radiation

doses on intrinsic viscosity of SA obtained are also shown in Figure 1. Intrinsic viscosity is 0.011948 cps, 0.011846 cps and 0.010548 cps for nonirradiated and irradiated at 5 kGy, 50 kGy dose on SA respectively.

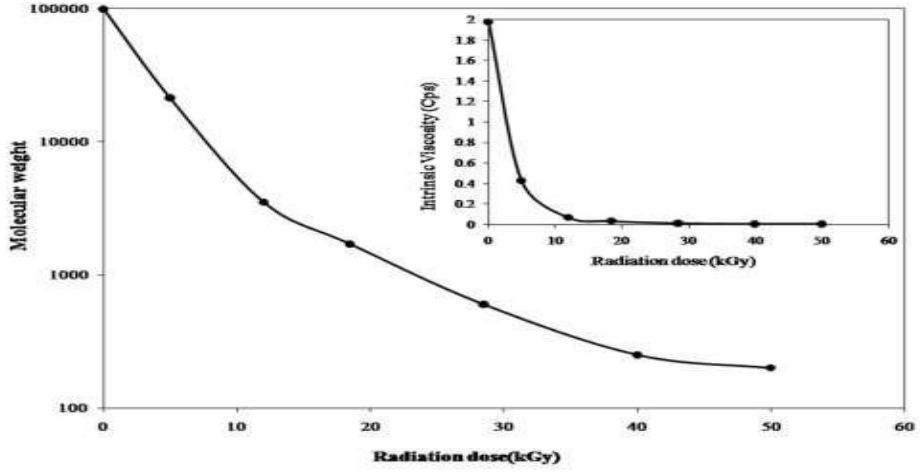

Figure 1. Changes in the viscosity average molecular weight of alginate powder after exposing to γ-irradiation at various radiation doses [7].

3.1.3 FT-IR spectroscopic analysis

Figure 2 shows the FTIR spectra of NSA solution and irradiated SA solution at 12 and 20 kGy. FTIR spectrum of NSA solution shows a broad band at 3395 cm^{-1} indicating the stretching vibration of aliphatic O-H group. Another peak at 2933cm^{-1} is from −C-H stretching vibration of $–CH_3$ group. Band at 1609 cm^{-1} represents the stretching vibration of the carbonyl group C=O. The existence of the peak at 1416cm^{-1}, is characteristic for the bending vibration of $–CH_3$. Peak at 1100 cm^{-1} belongs to the –C-O vibration of polysaccharide. For irradiated SA by 12 kGy and 20 kGy, peaks are obtained at almost similar regions. This indicates that only polymer is broken down into smaller molecules but the main unit structure is not broken.

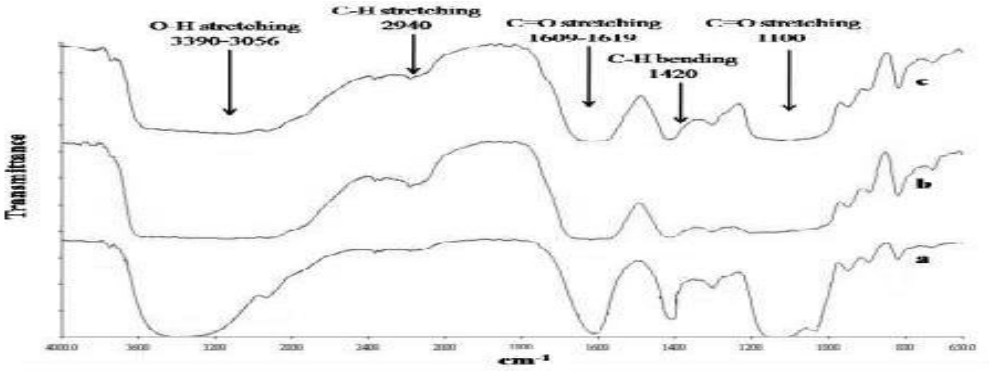

Figure 2. FTIR spectra of SA solution:a)NSA b) I2 kGy irradiated SA c) 20 kGy irradiated SA[7]

Peak at 1619 cm^{-1} for alginate was taken as the reference peaks due to the fact that carboxyl groups do not change after degradation. The spectrum of irradiated SA exhibited most of the characteristic adsorption peaks of nonirradiated solution but with some differences. For instance, the bands at 1619 cm^{-1} for carboxylate groups, at 3440 cm^{-1} for OH groups and at 1095 cm^{-1} and 1037 cm^{-1} for C-O stretching became broader and shift to another wave numbers. The spectra indicated the formation of new C=O and OH groups suggest the degradation due to radiation. SA irradiated by 12 kGy conditions lead to the scission of glycosidic bonds with the change of the structure of reducing end residue. This is manifested as an increase in the ratio of OH group peak and broad C=O peak to the NSA. Simultaneous decreasing of the peak ratio of C–O–C group to the NSA has been also noticed.

Based on the results of FT-IR spectral analyses of the tested irradiated SA, in this study and several schemes for the radiation degradation of polysaccharides which have been proposed by several authors, e.g., CS, pectin, carrageenans and alginate, a possible mechanism for the radiation degradation of SA can be proposed as follows (figure 3)[8-14].

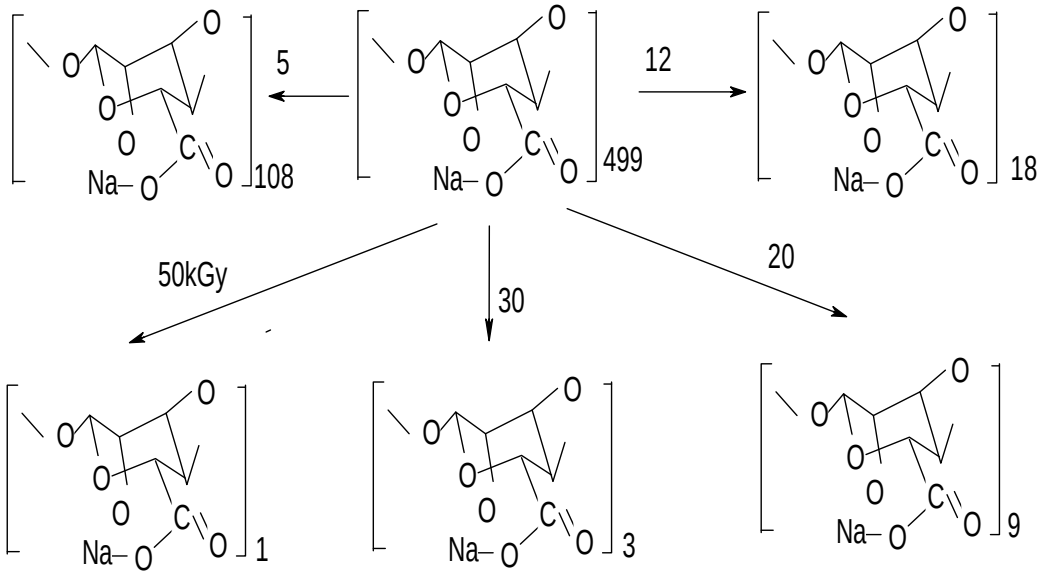

Figure 3. Mechanism for the radiation degradation of solid alginate (SA)

3.1.4 Irradiated SA as plant growth promoter on selective crops

Radiation processed polysaccharides are now being investigated as plant growth promoters by a number of researchers [11,12]. In recent studies, irradiated polysaccharides have mainly been applied in hydroponic or spraying systems for some specific type of crops only. Hence, more studies are desired to establish the optimum conditions for use of such materials as plant growth promoters. In this regard, the growth promotion activity of radiation depolymerized alginate has been investigated for on tomato, spinach, mung dal, jute, pumpkin, egg plant, green chilli,

red chilli, cabbage and cauliflower plants up to 30 to 90 days. The growth promotion activity of these plants was tested on various growth parameters which are averages of plants height, number of leaves and stems, leaves area, vine's thickness, root length, size of fruits, number of fruits, harvest time, first flowering, wet & dry weight yield. Solutions of SA irradiated at 12 kGy were applied by foliar spraying system for the growth promotion of selective plants.

3.1.5 Growth promotion effect on plants height

The figure 4 shows that the changes of plant height after 90 days due to applying nonirradiated and irradiated SA (at 12 kGy) of different concentration. An enhancement of plant height of tomato was observed at 500 ppm solution concentration and plant height of spinach, mungdal, jute, pumpkin, egg plant, green chilli showed best result at 300 ppm and red chilli plant showed best height after applying 1000ppm solution. The plants' height of tomato 57%, spinach 144%, mungdal 300%, jute 28%, pumpkin 167%, egg plant 300%, green chilli 183%, red chilli 120% better results showed than control. The best plant height was observed in mung dal and egg plant at 300% than that of control.

Foliar spray of alginate derived oligosaccharide SA could stimulate the synthesis of some antioxidative enzymes in a short period of time to catabolize reactive oxygen species (ROS), thus, protecting the cellular membranes from being damaged. Actually irradiation can modify the viscosity molecular weight, hydrophilic and mechanical properties of alginates resulting in enhance properties. SA also acts as signal molecules that regulate growth and development of the plant as well as defense reactions by regulating gene expression [5,13] and thus plant increase support happen.

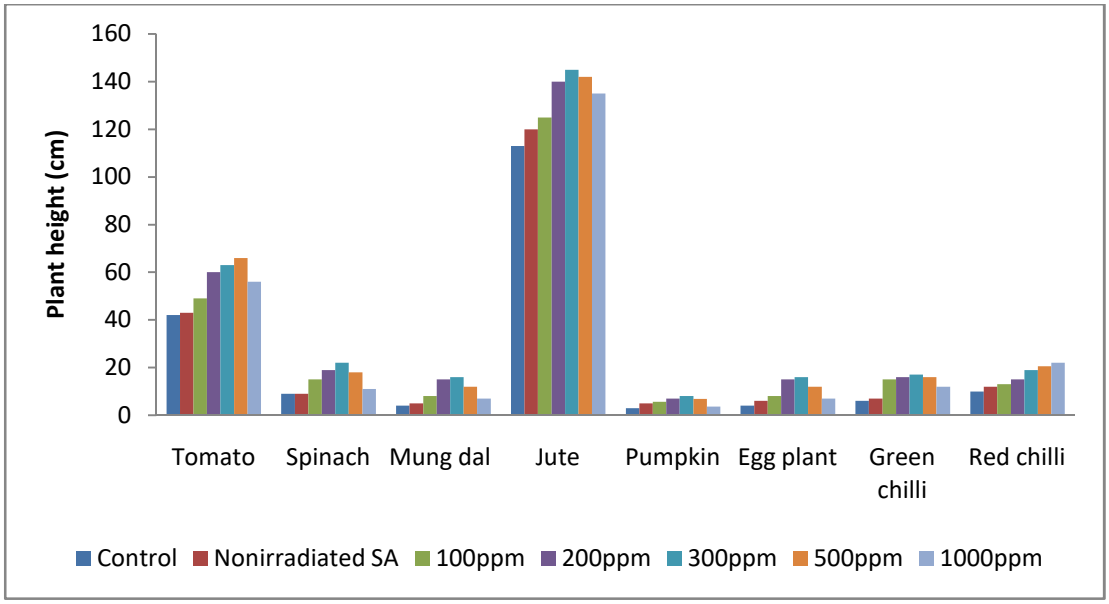

Figure 4. Growth promotion effect of SA and irradiated SA on selective crops' height on 90 days of foliar spray.

In the case of irradiated SA it is supposed that irradiated SA acts on the cell membrane in such a way that enzyme(s), or secondary messenger(s) [L (+) – adenosine] could trigger the cascading effect, which might result in increased plant metabolism and accumulation of various critical intermediary metabolic compounds, leading to improved growth of the plants.

3.1.6 Growth promotion effect on shoot growth

The figure 5 shows that the changes of shoot growth after 90 days due to applying nonirradiated and irradiated SA at 12 kGy of different concentration. It was found that an enhancement of shoot growth of tomato, mung dal, jute, egg plant and red chilli plant was observed best at 500 ppm solution concentration and 300 ppm concentration was found best to spinach, pumpkin and green chilli plant. The shoot growth of tomato 40%, spinach 16%, mungdal 100%, jute 150%, pumpkin 60%, egg plant 67%, green chilli 200%, red chilli 60% better results showed than control. The best shoot growth showed for green chilli at 200% than control.

Radiation, a convenient tool for degradation of polysaccharides, can be performed at room temperature and the degraded product obtained by this process can be used without any purification. Although SA can be depolymerised through enzymatic degradation but plant researchers suggest the use of radiation processing technology for degrading the polysaccharides [5,15]. Irradiated SA has several novel unique features that canbe useful in agriculture. In fact, polysaccharides, such as SA, undergo chain scission by irradiation. The irradiation of SA by gamma rays affects the overall polymer cross-linking process. Consequently, its application influences the biological properties of the plant cells [18]. However, the phenomenon which stimulates the processes related to promotion of plant growth still needs further investigations. In addition, various researchers have emphasized that irradiated SA could successfully act as a plant growth promoter and also a potent enhancer of the activity of various enzymes in the plants [16-21].

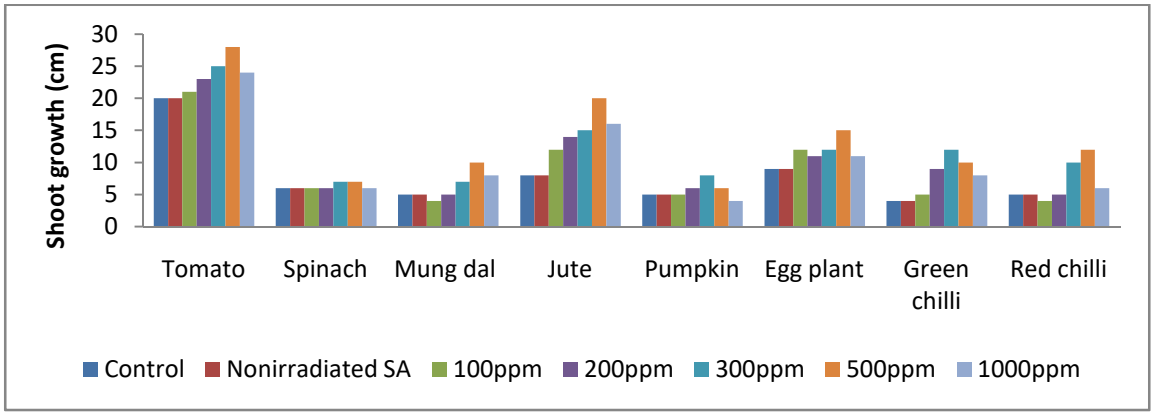

Figure 5. Growth promotion effect of SA and irradiated SA on selective crops' shoots growth after 90 days of foliar spray.

Cell division, enlargement and differentiation are the main processes that determined the quality and quantity of plant growth. These processes are affected by various internal and external factors including (a) supply and absorbtion of nutrients, which have critical importance in cell metabolism and (b) involvement of phytohormones/plant growth regulators for maintaining a healthy source-sink relationship. Plant growth regulators get involved through the modification of transcription, translation and/or differential sensitivity of the tissue.

3.1.7 Growth promotion effect on number of leaves

The figure 6 shows that the changes of number of leaves after 90 days due to applying nonirradiated and irradiated SA at 12 kGy of different concentration. An increased production of number of leaves of tomato was observed at 500 ppm solution concentration and for spinach, pumpkin, green chilli and red chilli plant shows best number of leaves after applying 300 ppm solutionthan control. The number of leaves of tomato 82%, spinach 86%, pumpkin 110%, green chilli 246%, red chilli 300% greater number of leaves showed than control. The greater number of leaves found on red chilli at 300% than control.

The leaf analysis of Mentha arvensis and Cymbopogon flexuosus revealed enhanced N, P and K contents due to irradiated SAapplication. In this regard, irradiated SA appears to facilitate the efficient absorption and utilization of mineral nutrients [22, 23]. Similar enhancement in leaf N, P and K contents due to irradiated SA application to various other crops has been reported by [24-25]. They applied various concentrations of irradiated SA on the leaves of Papaver somniferum L. and reported that irradiated SA at 120 ppm concentration improved leaf-nitrogen content to the maximum.

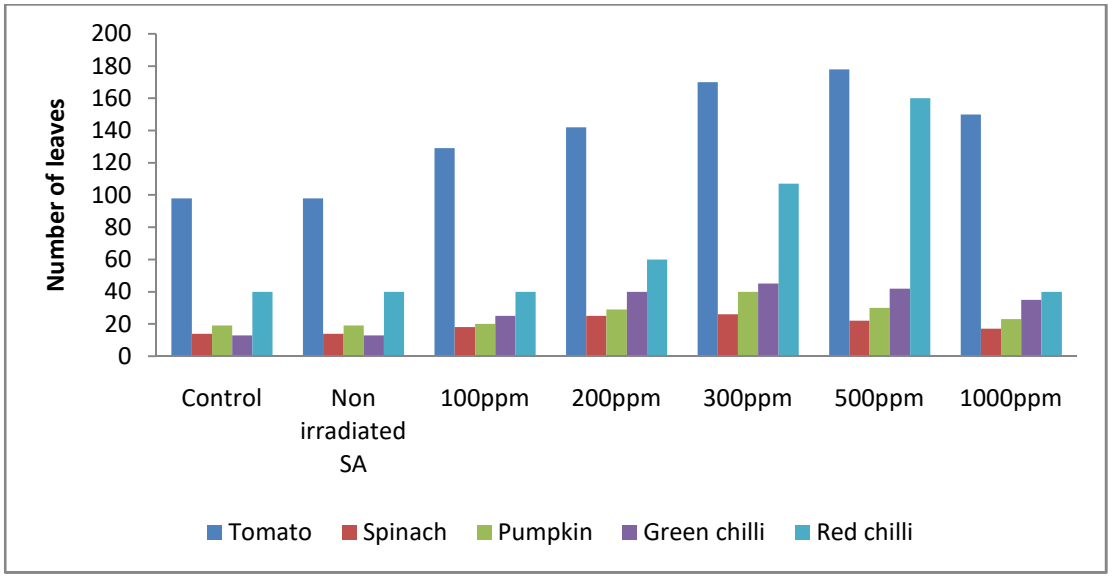

Figure 6. Growth promotion effect on number of leaves of selective crops' after 90 days of foliar spray.

3.1.8 Growth promotion effect on leaves area

The figure 7 shows that the changes of leaves area after 90 days due to applying nonirradiated and irradiated SA at 12 kGy of different concentration. It was observed that an increased leaves area of tomato was observed at 500 ppm solution concentration and for spinach, jute, pumpkin plant shows best leaves area after applying 300 ppm solutionthan control. The leaves area of tomato 51%, spinach 86%, jute and pumpkin 110% increased than that of control. The increased leaves area was observed 110% for pumpkin than control. The increased leaves area was observed 110% for pumpkin than control.

The applied of growth promoters had started foliar growth after 7 days of seeding. The increased content of photosynthetic pigments due to irradiated SA application on leaves in this study could also be attributed to possible the increase in number and size of chloroplast, the amount of chlorophyll per chloroplast and proper gramma development. This perception is further confirmed by the correlation studies, wherein leaf-nitrogen content was positively correlated with total content of chlorophyll and carotenoids. It was revealed that the irradiated SA enhanced N content in leaves. It is well known that nitrogen markedly promotes the synthesis of active photosynthetic pigments [23]. A promotive effect of irradiated SA on chlorophyll content might perhaps be attributed to the irradiated SA increased leaf-N that is a constituent of chlorophyll molecule. Irradiated SA also increased the total chlorophyll and carotenoids contents in the leaves of fennel, opium poppy, and beetroot [24- 26].

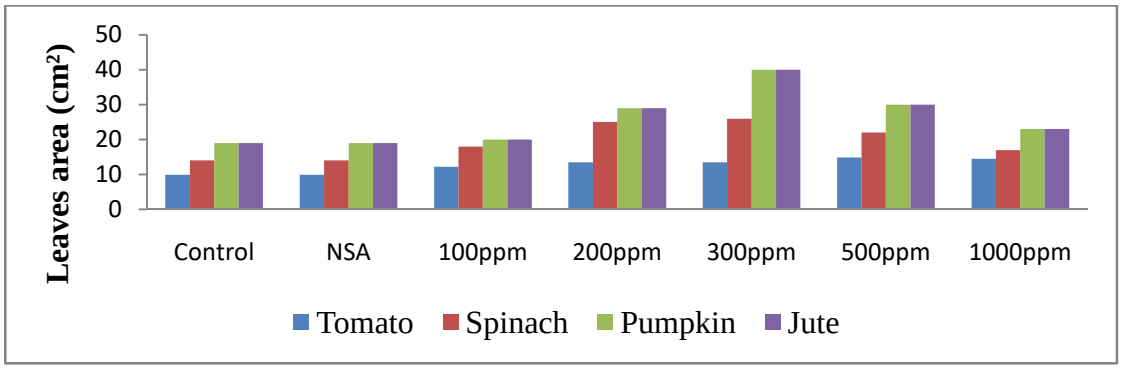

Figure 7. Growth promotion effect on leaves area of selective crops' after 90 days of foliar spray.

3.1.9 Growth promotion effect on number of fruits

The figure 8 shows that the changes of number of fruits after 90 days due to applying nonirradiated and irradiated SA at 12 kGy of different concentration. An enhancement of number of fruits of tomato, green chilli, red chilli and egg plants was observed at 500 ppm solution concentration and all concentration proved the better than control after applying irradiated SA. The productivity of tomato 214%, red chilli 120%, and

green chilli 300% and egg plant 80% increased than control. The productivity of green chilli showed 300% greater than control.

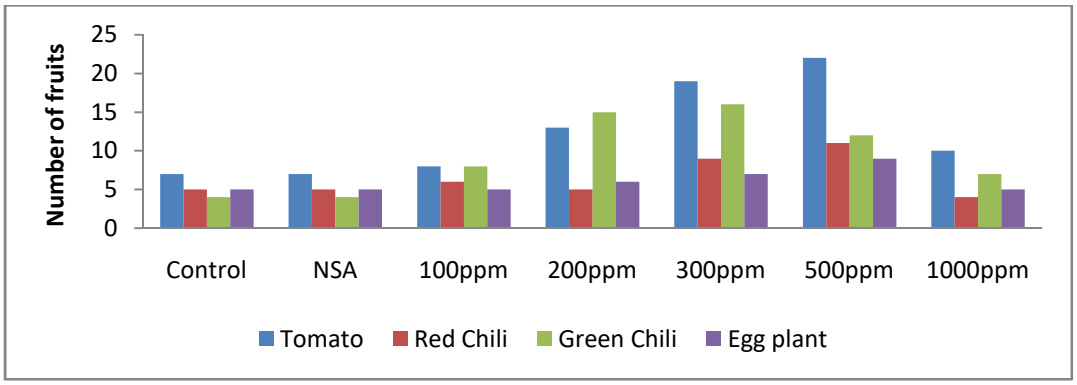

Figure 8. Growth promotion effect on production of selective crops' after 90 days of foliar spray.

Enhancement in leaf-nutrients, particularly N, due to irradiated SA application, could be attributed to the compositional or chemical change in plants leading to alterations in nitrogen concentration. Presumably, increased uptake of nutrients enhanced the photosynthesis and improved translocation of photosynthates and other metabolites to the sinks that might presumably contribute to the improved yield of irradiated SA treated plants [27, 28].

As a rate limiting photosynthetic enzyme, it regulates the synthesis of carbon compounds which are transported to the sink organs and are utilized for sustaining growth and development of fruits.

3.1.10 Growth promotion effect on size of fruits and weight

Figure 9 showed in below represents the sizes of fruits (diameter of fruits in cm^2) of different plants after 90 days due to applying of nonirradiated and irradiated SA of 100 to 1000 ppm concentration. Sample ID for figure 10 represents in table 1.

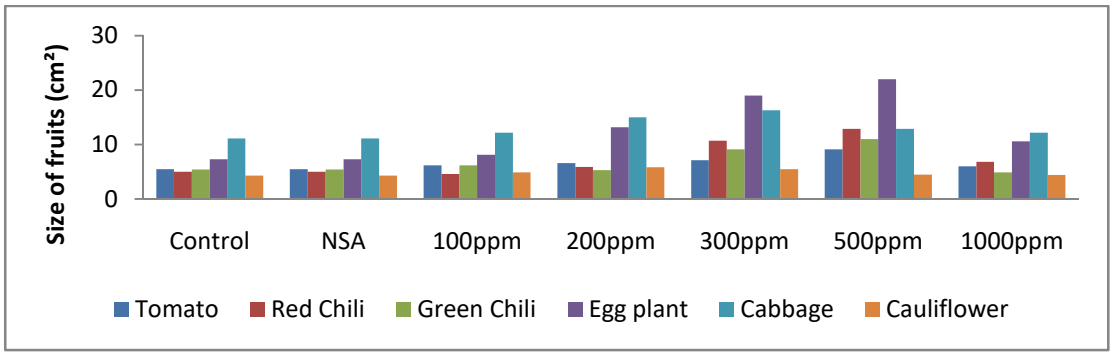

Figure 9. Growth promotion effect on sizes of fruits of selective crops' after 90 days of foliar spray

Figure 10 showed the photograph of control and irradiated SA applied tomato (figure 10a, 10b), cabbage (figure 10c, 10d) and cauliflower (figure 10e, 10f) plants along with their fruits. It has also noticed from the photograph that the size and weight (figure 10) of the fruits were also increased after the treatment applied. The size of tomato increased 66%, red chilli 158%, green chilli 103% egg plant 201%, cabbage 47% and cauliflower 35% greater than control. The weight of tomato increased 315%, red chilli 216%, green chilli 17%, pumpkin 127%, jute 50%, spinach 144%, mung dal and egg plant 300%, give up than control. The size of fruits increased 201% in egg plant than control.

Figure 10a	Figure 10b
	
Figure 10c	Figure 10d
	
Figure 10e	Figure 10f
	

Figure 10. Growth promotion effect on fruit's size of selective crops after 90 days of foliar spray.

Table 1: Sample ID for figure 10

a. Tomato (control)	b. Tomato (500 ppm treated)
c. Cabbage (control)	d. Cabbage (300 ppm treated)
e. Cauliflower (control)	f. Cauliflower (500 ppm treated)

3.1.11 Growth promotion effect on fruit's weight

The figure 11 shows that the weight gains of fruits after 90 days due to applying nonirradiated and irradiated SA at 12 kGy of different concentration. Increased weight gains on tomato was observed at 500 ppm solution concentration and for spinach, mung dal, jute, pumpkin, egg plant, green chilli and red chilli plant shows best weight gain after applying 300 ppm solution than control. The weight of tomato increased 315%, red chilli 216%, green chilli 17%, pumpkin 127% and egg plant 300%, give up than control. The weight of fruits increased in tomato at 315% than control.

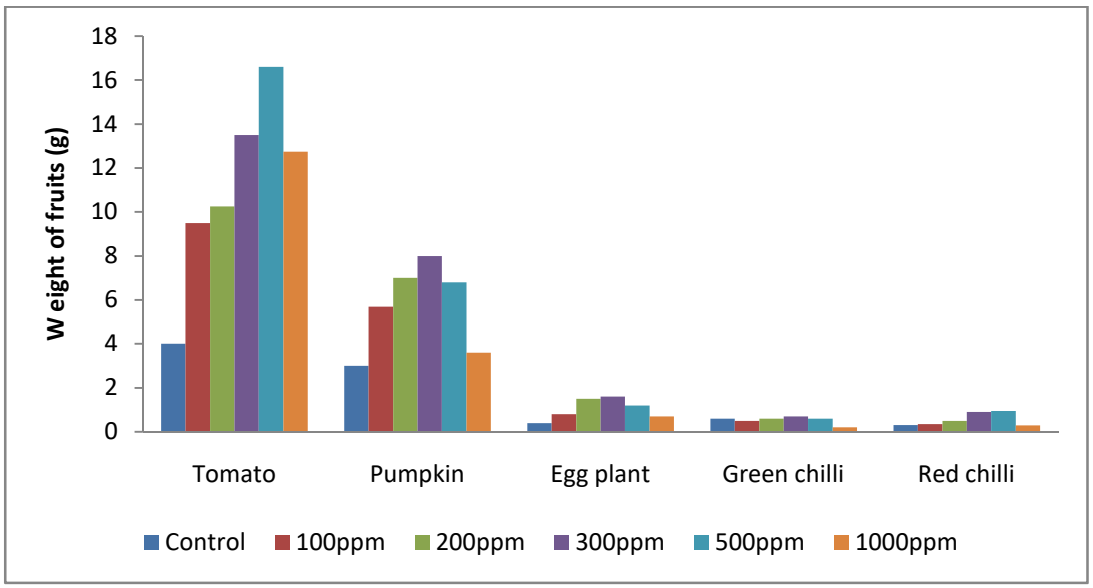

Figure 11.Growth promotion effect on fruits' weight of selective crops after 90 days of foliar spray

The enhanced values of yield attributes with spray of irradiated SA might be ascribed to the role of irradiated SA in plant growth in general [16,17]. Like irradiated SA, various other plant growth regulators have been used to improve the yield and quality of essential oil of these crops. Increasing concentrations of growth regulators such as folic acid (25 and 50 ppm), ethephon (50 and 100 ppm) and irradiated AA (lAA,100 ppm) has, for example, been reported to increased the percentage of dihydrocarvone content in the essential oil of dill. In addition, the oil content and yield were elevated by kinetin (20 mg per litre) application indicating that affected target at the terminal enzymic transformations in the carvone pathway from limonene.

3.1.12 Growth promotion effect on root length

The figure 12 shows that the increased root length of selective plants' after 90 days due to applying of NSA and different concentration of 12 kGy irradiated SA. It was observed that increased root length of tomato were found at 500 ppm solution concentration and for spinach, mungdal, jute, pumpkin, egg plant, green chilli and red

chilli plant shows best root length after applying 300 ppm solution than control. Figure 13 showed the photograph of control and irradiated SA applied tomato (figure 13a), red chilli (figure 13b) and green chilli (figure 13c) plants along with their roots. It was observed from figure 13a that root of tomato plants increased upto 21cm applying 500 ppm irradiated SA than that of control. Thus almost 162% increased root indicated the remarkable growth of tomato plants. The root length of tomato increased 162%, spinach 145%, mung dal 255%, jute 50%, egg plant 300%, red chilli 143% pumpkin and green chilli showed 127% lengthy than control. Egg plant showed the best increased root length of 300% than control. Sample ID for figure 13 represents in table 2.

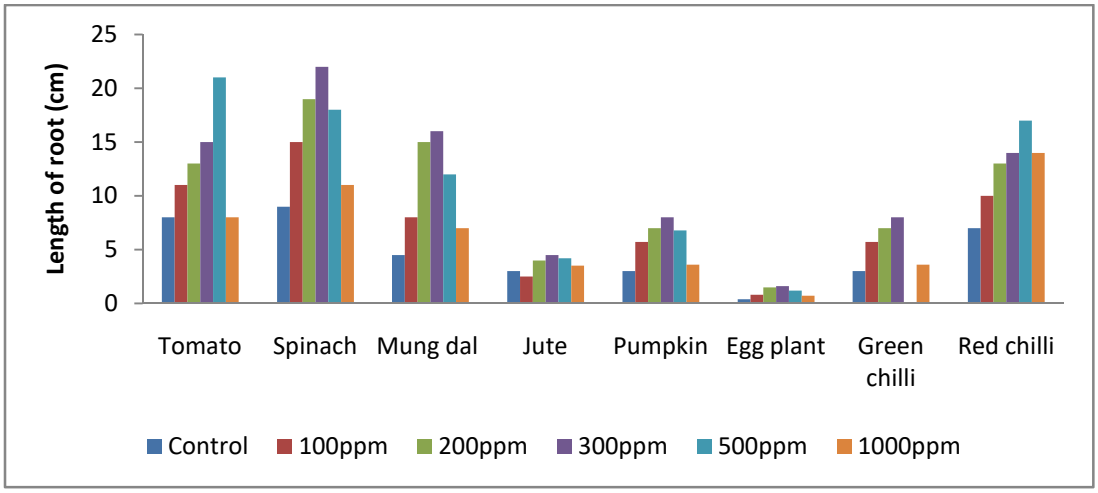

Figure 12.Growth promotion effect on root length of selective crops after 90 days of foliar spray.

Plant requires an adequate supply of nitrogen in order to synthesize amino acids, proteins, nucleic acids and other cellular constituents necessary for their growth and development. The first step of nitrate assimilation involves the reduction of nitrate to unstable nitrite by nitrate reductase (NR), which is the rate-limiting enzyme in nitrogen assimilation and akey enzyme of metabolic regulation. Nitrate reducing power of the plant is one of the important factors determining the plant growth. However, the process of nitrate reduction is directly or indirectly dependent on the metabolic sensors and/or signal transducers [29]. The level of the enzyme is dependent on a number of factors, boom within or outside the plants. One of the major regulatory factors, determining the activity of NR, is the level of endogenous phytohormones per se or those added from outside [29]. There might be significant improvement in NR activity at all the five stages of sampling due to irradiated SA application compared with the Control.

Tomoda et al. (1994) [17] revealed that depolymerised alginate had growth promoting effects on the elongation of barley root, especially that of the radical. They observed the effective concentration of alginate (100-300 µg/L) for elongation of roots with no inhibition at the highest concentration. In another study [30], use a mixture of

Figure13a.

Figure 13b.

Figure 13c.

Figure13d.

Figure 13. Photograph of the growth promotion effect on root length of selective crops' after 90 days of foliar spray.

Table 2: Sample ID for figure 13

a. Image of tomato plant's root (control)	b. Image of tomato plant's root (500ppm SA treated)
c. Image of the roots of red chilli plants (300ppm SA treated)	d. Image of the roots of green chilli plants (300ppm SA treated)

oligosaccharides of SA, showed growth promoting activity of SA regarding elongation of lettuce root at a concentration range of 200-3000 µg /mL. In the light of above facts, it was concluded that alginate acts as an endogenous elicitor-like substance [31].

3.1.13 Growth promotion effect on reduction of disease

Effect of irradiated SA on reduction of diseases was investigated and their data is represents in figure 14 and 15. To detect the disease control; the affected leaves were counted after every 7 days. The numbers of affected leaves were observed more in control sample than treated sample. Figure15 showed the photograph of control and irradiated SA applied plants along with affected leaves. On the last day of experiment the affected leaves on control tomato, jute, green chilli, mung dal, and pumpkin plant having ±10 whereas on treated plants there have ± 2. Spinach plants treated by 300 ppm showed 100% potent action against disease. Sample ID for figure 15 represents in table 3.

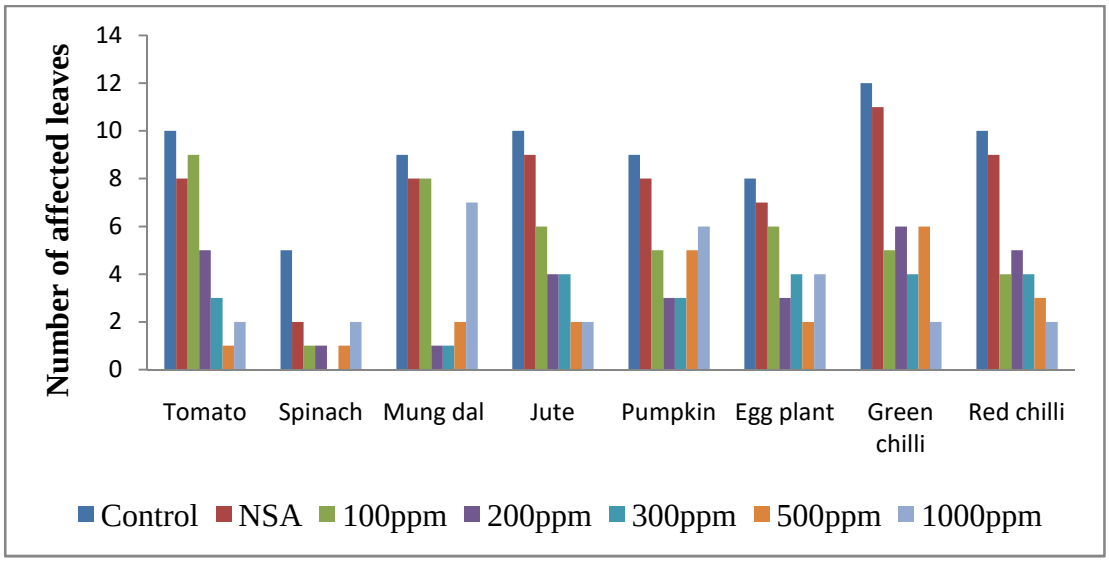

Figure 14. Growth promotion effect on reduction of disease of selective crops after 90 days of foliar spray of nonirradiated and irradiated SA.

Table 3: Sample ID for figure 15

15 a: Control red chilli plants	15 b: 100 ppm SA treated red chilli plants
15 c: 200 ppm SA treated red chilli plants	15 d: 300 ppm SA treated red chilli plants
15 e: 500 ppm SA treated red chilli plants	15 f: 1000 ppm SA treated red chilli plants

The role of irradiated SA as disease control in plant is well known. During cultivation tomato vine is very much affected by various forms of mildew and blight disease and some common tomato pests named stink bugs, cutworms, tomato hornworms and

Figure 15: Photograph on reduction of disease of red chilli plants after 90 days of foliar spray nonirradiated and irradiated SA.

tobacco hornworms, aphids, cabbage loopers, whiteflies, tomato fruit worms, flea beetles, red spider mite, slugs,[32] and colorado potato beetles.

3.1.14 Observations

- After 90 days of foliar spray of nonirradiated and irradiated SA on selective crops (tomato, spinach, mungdal, jute, pumpkin, egg plant, green chilli, red chilli) the best plant height were observed in mung dal and egg plant at 300% than that of control.
- The best shoot growth showed for green chilli at 200% than that of control.
- The greater number of leaves found on red chilli at 300% than that of control.
- The increased leaves area was observed 110% for pumpkin than that of control.
- The productivity of green chilli showed 300% greater than that of control.
- The size of fruits increased 201% in egg plant than that of control.
- The weight of fruits increased 315% than that of control.
- The root length increased in egg plant 300% than that of control.
- Spinach plants showed 100% potent action against disease after 90 days foliar spray of 300 ppm irradiated SA.
- After 12 kGy radiation dose, molecular weight of SA was achieved 5000 Da. Spinach, Pumpkin, Mung dal, Eggplant, Tomato, Green chilli, Red chilli and Jute showed increase of plant height, number of leaves, leaves area, shoot growth and root length of them after the SA application. Significant reduction of diseases was observed on those crops after the SA application.
- In treated plants the absorption of irradiated SA acted as a growth promoter, disease control activity which resulted in elongation of plant root and shoot growth, increase in plant productivity and an improvement in physiological parameters compared with the untreated plants and could act as a plant growth promoter.

3.1.15 References

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Section 3.2: Application of irradiated SA on betel vine

3. Results & Discussion

Section 3.2: Application of irradiated SA on betel vine

3.2.1 Introduction

Figure 1 shows the photograph of trial piper betel vine's garden, which located in Cox's bazar District, Bangladesh. Some cultivators were cultivating betel vine by their personal funding. It to be found under the base of 150ft surrounded high hill area. They work hard for irrigate due to less water getting condition. Some researcher reported on comparative study of some soil properties in forested and deforested areas in Cox's Bazar and Rangamati Districts, Bangladesh, The study dealt with the assessment of impact of deforestation on soil through a comparative analysis of soil physicochemical properties of natural forest of Cox's bazar and deforested areas of Rangamati. Soil samples from three depths (top, middle and bottom) under natural forest and nearby deforested areas were collected to investigate soil properties. Forest soils show no significant change in particle size distribution. Bulk density of forested soils shows the significant differences in top and middle layers. Soil p^H in top and middle soil, organic matter in top soil and available phosphorus in middle soil of the forest site are found to be significantly higher than that of the deforested soils. Forest soils also have significantly higher level of exchangeable Ca^{2+}, K^+ in top and middle soil and Mg^{2+} at all depth than those of deforested site. Exchangeable Na^+ and cation exchange capacity (CEC) are observed unchanged in both sites. The results suggest that change in soil properties was more obvious in surface and sub surface portions of both areas [1].

Gamma irradiation is useful for degradation of sodium SA and CS. The lower molecular weight of SA and CS promote higher growth of plants. The irradiation dose required for degradation of CS derivatives is low. The water radiolysis products like •OH radicals have a great effect on positive degradation of CS derivatives. The irradiation in solution form is better than that in solid form which reduces the required dose for degradation. Polysaccharides are typical degradable materials under ionizing radiation through the β-(1–4) glycosidic bond cleavage resulting in the reduction of their molecular weights. The reason is due to the formation of hydroxyl radicals (•OH) through the radiolysis of water and as illustrated in the following equations

H_2O + γ-ray $^-$•OH, e^- (aq), •H----------(Equation 1)

In general hydroxyl radical reacts with carbohydrates exceedingly rapidly, abstracting a C-bonded H atom according to the general equation:

R-H + HO• $^-$ R• + H_2O---------- (Equation 2)

These radicals then undergo further reactions before ending up as products as follows:

$R\bullet\ (C_1, C_6)\text{–}F_1\bullet + F_2$ (scission) -------(Equation 3)

Where R-H polysaccharide macromolecules, R• (Cn) and F_1•, F_2 are fragments of the main chain after scission

Piper betel Linn., (family Piperaceae) commonly known as the betel vine is a important medicinal and recreational plant in Southeast Asia. The most probable place of origin of betel vine is Malaysia but today the plants are also cultivated in Bangladesh, India, Srilanka, Burma and Nepal [2, 3]. Betel vines are one of the highly investigated plants and phytochemical studies show that betel vine contains a wide variety of biologically active compounds whose concentration depends on the variety of the plant, season and climate. The aroma of betel leaf is due to the presence of essential oils, consisting of phenols and terpenes [4]. Chewing betel leaf is supposed to prevent bad breath (halitosis), improve the vocalization, harden the gum, conserves the teeth and sweetens breath. The infusion prepared from the leaves and stems are supposed to be useful in treating indigestion, bronchitis, constipation, congestion, coughs and asthma. The leaf juice is given systemically to treat cough and indigestion in children. The Essential oil isolated from the leaves is supposed to be useful in treating respiratory catarrhs and as an anti-septic [4].

The aim of the present study was to investigate whether the foliar application of irradiated SA could be used to enhance the growth, physiological activities, yield attributes and other active constituents in Piper betel L.

Figure 1. Photograph of the experimental betel vine garden in Cox's bazar

Irradiated SA was used on betel vine (Piper betel Linn.) to evaluate its plant growth promotion activity. SA solutions were treated at different (5-50 kGy) radiation doses and optimized in terms of the molecular weight. A range of concentrations of SA were applied through foliar spraying. The effects of various concentrations ofirradiated SA on betel vine were determined in terms of various growth attributes and plant nutrients. The plant showed insignificant changes for all the studied attributes after treatment of NSA on it and for the control plant.

3.2.2 Growth promotion effect of irradiated SA on plant height, number of leaves, leaf area and shoot growth

Comparative growth parameters of the betel vine plant specimens after using various concentrations of SA solution at 12 kGy are shown in table 1 and table 2. It is apparent that by applying irradiated SA at higher doses, showed significantly higher plant growth and works as a stimulator for betel vine.

The results obtained by treatment with irradiated sodium alginate showed increased plant height (shown in table 1). The figures represent the effects of irradiated sodium alginate on betel vine plants. It exhibits that, plant height were maximum at 500 ppm, 12 kGy irradiated SA treated betel vines after the 30 days foliar spraying at 7 days intervals, but it reduces at both very low (350 ppm) and very high (750 ppm) concentration.

After 30 days of foliar spray of nonirradiated and irradiated SA on betel vine plant control plants increased their height of 37% and NSA, 350 ppm, 500 ppm and 750 ppm treated betel vines increased 44%, 48%, 72%, and 57% respectively than control. Betel vine treated with 500 ppm SA showed the best plant height of 72%.

The table 1 also represents the effects of irradiated sodium alginate on betel vine's number of leaves raise. It exhibits that, the effectiveness of 12 kGy irradiated sodium alginate has increased the leaves count compared to untreated samples. It also shows from this table that, leaves numbers were maximum at 500 ppm concentration of sodium alginate and chitosan solution. After 30 days of foliar spray of nonirradiated and irradiated SA on betel vine plant control plants increased their number of leaves of 22 and NSA, 350 ppm, 500 ppm and 750 ppm treated betel vines achieve 24,26,43,30 leaves respectively than control. Betel vine treated with 500 ppm SA showed the best leaves production than control.

Higher number of leaves contribute to a more photosynthesize production of the plants and this is what has been observed in this study. The plant height and number of average leaves nourished with only water and chemical fertilizer (in case of control and the NSA sample) is lower than the plant treated with irradiation.

Table 1: The effect on plant height and number of average leaves of betel vine after application of nonirradiated and irradiated SA solutions for different days.

Plant height of betel vine							**Number of average leaves**				
Sample ID	**Day 0**	**Day 7**	**Day 15**	**Day 21**	**Day 30**		**Day 0**	**Day 7**	**Day 15**	**Day 21**	**Day 30**
Control	88	90	99	106	120		5	6	9	15	22
NSA	87	93	99	107	125		5	6	10	16	24
350 ppm	88	94	107	110	130		5	6	11	17	26
500 ppm	85	99	120	137	146		5	6	16	25	43
750 ppm	86	95	105	112	135		6	7	12	20	30

The results obtained by treatment with irradiated sodium alginate showed increased leaves area and shoot growth (shown in table 2). It exhibits that, leaves area were maximum at 500 ppm as compared to all other concentration and control. After 30 days of foliar spray of nonirradiated and irradiated SA on betel vine plants NSA, 350 ppm, 500 ppm and 750 ppm treated betel vines increased their area of leaves of 30%, 59%, 60%, 100% respectively than control. Betel vine treated with 750 ppm SA showed the best leaves area of 100%. Shoot growth increased the best at 350 ppm SA treated betel vines of 250% than control.

Table 2: Effect on leaf area and number of shoot growth of betel vine after application of nonirradiated and irradiated SA solutions for different days.

Maximum leaf area average (cm^2)							**Number of shoot growth**				
Sample ID	Day 0	Day 7	Day 15	Day 21	Day 30		Day 0	Day 7	Day 15	Day 21	Day 30
Control	27	50	75	88	100		0	0	1	2	2
NSA	25	50	80	92	130		1	1	2	2	3
350 ppm	22	30	138	156	159		1	2	3	4	7
500 ppm	22	27	131	157	160		1	1	2	3	6
750 ppm	22	28	90	162	200		0	0	1	2	4

In all cases, irradiated and nonirradiated SA had a stimulatory effect on leaf area, length of roots and newly developed shoots, than controlled one (table 1, 3). The results clearly demonstrated that irradiated SA effectively stimulates the developing of roots and shoots of plants. Oligosaccharides derived from depolymerization of alginates (referred to as oligoalginates) have novel features such as stimulation of growth of bifidobacteria, promotion of germination and shoot elongation of plants [5-7]. Biologically active oligosaccharides act as signal molecules that regulate growth of the plant as well as defense reactions by regulating gene expression [8].

3.2.3 Growth promotion effect on root's length and thickness

Figure 2 shows the root thickness and root length of betel vine as a function of different concentration of SA at 12 kGy irradiation dose. It is apparent that irradiated SA has higher root thickness and root length than the control and sample nourished by NSA.Comparing the results, the highest root length (17cm) and thickness (7.6 mm) was found by the treatment of 500 ppm solution concentration of irradiated SA. Whereas it was found 13.0 cm and 5.1mm for control sample and 12.9 cm and 5.8 mm was found for plant treated with NSA solution. The best effects on root length and thickness were increased at 31%, 49% for 500 ppm SA treated betel vine as compared to the control plants.

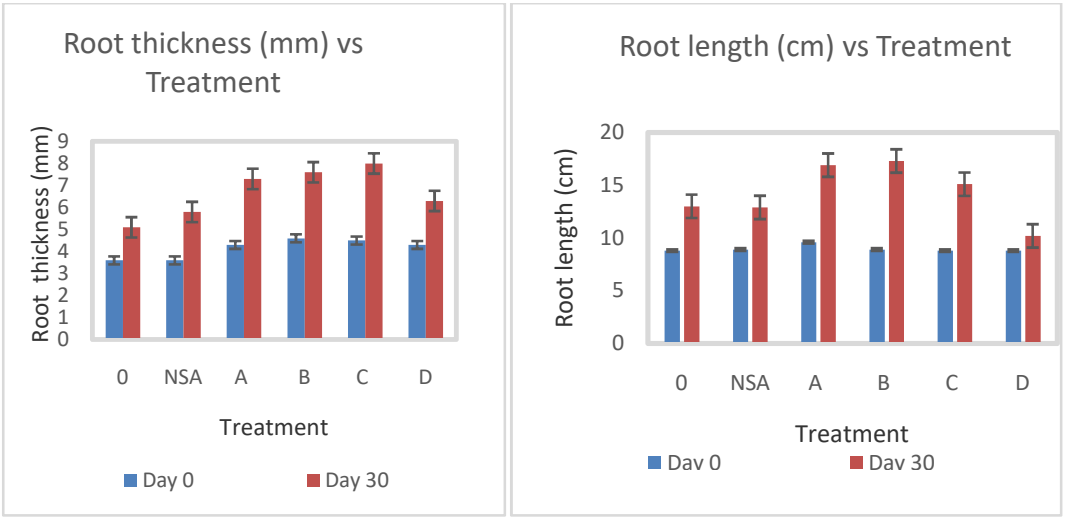

Figure 2. Effect on root thickness and root length of betel vine after application of nonirradiated and irradiated SA solutions on 30 days of foliar spray.

3.2.4 Growth promotion effect on fresh weight of leaves

Effect on fresh weight of leaves of betel vine after application of nonirradiated and irradiated SA solutions for 30 days represents in table 3. In this analysis, a positive effect of irradiated SA on plant growth of betel leaves is observed.

Table 3: Effect on fresh weight of leaves of betel vine after application of nonirradiated and irradiated SA solutions for different days.

Treatment	Plucked leaves from 10 plants	Total weight, (g)	Weight gained, (%)
O (Control)	10	20	100%
NSA	10	22	11%
350 ppm	23	61	200%
500 ppm	24	79	288%
750 ppm	24	57	178%

It is experimental that (table 3) irradiated SA has significantly higher fresh weight of leaves compared to that of the plant treated by NSA or the control specimen. Highest amount (79g) of fresh betel leaves were obtained from betel vine plants treated with the SA solution of 500 ppm concentration at 12 kGy irradiation doses. This is an increase of 288% in the fresh weight of betel leaveswith compared to control. Researchers observed that sodium salt of AA or irradiated AA increased the essential oil and menthol content in case of Mentha piperita [9-11]. In this study, a similar result has been observed for betel leave's fresh weight.

3.2.5 Nutrient uptake in betel vine

Nutrient consumption in betel leaf is shown in table 4. From the table it has been observed that Fe and Ca uptake in the plants increased significantly at 750 ppm after irradiated SA treatment. However, Na and K and Zn content decreased in the leaves after the treatment applied.

Alginate is an excellent chelator of many metal ions (e.g. Cu, Ca, Ni, Co, Pb, etc.). Thus it absorbs Fe and Ca ions by forming metal complex and retain on the leaf. Zn possesses relatively low ligand field stabilization energy and due to this their concentration has moderately changed after the treatment. Na and K ions do not take part in the complex formation rather they go to the solution and this is why the concentration of them decreased in the leaf. After the 30 days foliar spraying of 750 ppm SA on betel vines, Fe and Ca increases 110 % and 31% respectively than control leaves

Table 4: Effect on nutrient content of betel vine after application of nonirradiated and irradiated SA solutions for 30 days.

ID	Fe (mg/kg)	Zn(mg/kg)	Na(mg/Kg)	K(mg/Kg)	Ca(mg/Kg)
Control	3.80±0.38	3.82±0.01	21.95±0.86	1418.87±17.03	134.36±1.88
NSA	2.00±0.17	1.42±0.01	10.00±0.01	400.19±3.44	134.3±2.75
350 ppm	2.02±0.17	1.97±0.01	10.42±0.01	430.17±3.44	275.00±2.75
500 ppm	3.53±0.05	3.44±0.01	14.58±0.50	859.33±18.05	263.33±5.00
750 ppm	7.95±0.06	3.34±0.02	14.38±0.01	1222.74±15.90	344.72±4.14

Irradiated SA might increase membrane permeability as generally noted in the case of plant growth regulators like GA3 [12-14] An increase in membrane permeability would in turn facilitate the absorption and utilization of mineral nutrients and the transport of assimilates [15-17]. This would also contribute toward enhancing the capacity of biomass production by plants as reflected by the increase in fresh and dry weights of plants in the present investigation.

3.2.6 Heavy metals consumption in betel leaf

From table 5 it has shown that, betel leaves contain insignificant amount of various heavy metals. From the table it has been observed that Pb uptake in the plants increased insignificantly at 500 ppm and 750 ppm after irradiated SA treatment. As, Hg, Cr and Cd consumption in betel leaves is less than 0.1, 0.3, 0.2 0.23 respectively. In 350 ppm treated betel leaves, Pb consumption decreases 96% than control.

Table 5: Effect on heavy metal content of betel vine after application of nonirradiated and irradiated SA solutions for 30 days.

Sample ID	Pb (mg/kg)	Cd (mg/kg)	Cr(mg/Kg)	As(mg/Kg)	Hg(mg/Kg)
Control	3.17±0.03	0.22±0.003	0.010±0.010	<0.1	<0.3
NSA	3.10±0.022	0.23±0.012	0.020±0.010	<0.1	<0.3
350ppm	1.62±0.005	<0.1	<0.1	<0.1	<0.3
500ppm	4.20±0.05	0.23±0.009	0.20±0.019	<0.1	<0.3
750ppm	4.19±0.14	0.23±0.006	0.20±0.022	<0.1	<0.3

3.2.7 Nutrient and heavy metal uptake by soil

Effect on nutrient and heavy metal content in soil of betel vine garden after application of nonirradiated and irradiated SA solutions for 30 days were represents in table 6. Hg consumption is less than <0.1 mg/kg both in soil taken near the SA treated betel vines and far from the treated betel vines. Increased As and Cd consumption value were found at 2.14 mg/kg and 1.04 mg/kg respectively in soil taken far from the treated betel vines. Zn, Fe increases but Ca, Na, K decreases in in the soil of treated area.

Table 6: Effect on nutrient and heavy metal content in soil of betel vine garden after application of nonirradiated and irradiated SA solutions for 30 days.

Parameter	**Unit**	**Technique used**	**Soil taken near the treated area**			**Soil taken out of the treated area**		
Zn	mg/kg	FAAS	27.624	±	0.221	32.70256	±	0.0654
Fe	mg/kg		13245	±	66.224	26516.18	±	79.549
Ca	mg/kg		1070.9	±	1.0709	349.76	±	2.0986
Na	mg/kg		102.77	±	5.7552	43.0642	±	4.2634
K	mg/kg		948.3	±	3.7932	841.3914	±	4.207
Cr	mg/kg		16.43	±	0.0986	18.581	±	0.0929
Pb	mg/kg		24.657	±	0.1726	31.4784	±	0.063
Cd	mg/kg		0.8946	±	0.0152	1.04928	±	0.0094
As	mg/kg	HGAAS	1.091	±	0.024	2.14228	±	0.0578
Hg	mg/kg	CVAAS	<0.1			<0.1		

The effect of heavy metal toxicity on the growth of plants varies according to the particular heavy metal involved in the process. For metals such as Pb, Cd, Hg, and As which do not play any beneficial role in plant growth, adverse effects have been recorded at very low concentrations of these metals in the growth medium. From this study we also found beneficiary effect by the small increases of heavy metals by betel vine.

3.2.8 Reduction of disease

On the last day of the experiment's deseased leaves were counted on treated and control vines. The results obtained from presents study revealed that 350 ppm irradiated alginate is causing complete inhibition of disease control almost 100% after 30 days foliar spraying of irradiated SA on betel vine plants than control. Figure 3a and 3b shows the photograph of desease affected control betel vines and desease free SA treated healthy betel vines.

3a. Disease affected betel vines (control)

3b.Disease free SA treated healthy betel vines

Figure 3. Photograph of the experimental betel vine garden after 30 days foliar spraying of irradiated sodium alginate on betel vine.

3.2.9 Anti-fungal test

The betel vine plant farmer's faceing heavy loss due to leaf rot of pan caused by var. Fungus was counted in various petri plates after incubation. Total numbers of fungi were calculated by colony counter in PDA media. Anti-fungal activity of radiation

processed SA on betel vine were represents in figure 4 it is clear that, at 12 kGy radiation doses 350 ppm sodium alginate solution concentration shows maximum antifungal activity in contrast with the other concentration and control plants.

For control the total fugal count was 462.6×10^2, which is reduced to 88.02×10^2 when plants are treated with nonirradiated sodium alginate (NSA). If we consider 750 ppm concentrated alginate treatment, we found that, with the 350 alginate concentration, total fungal count was decreased. From figure 4, fungal counts for 12 kGy sodium alginate solution at different concentrations are ranked as follows:

Control > NSA>750ppm >500ppm >350ppm

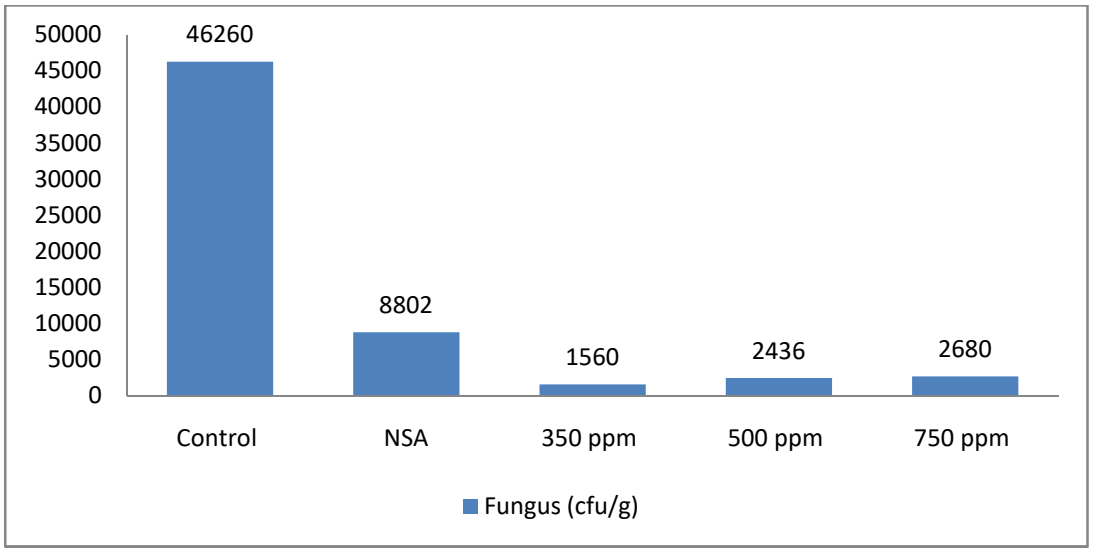

Figure 4. Anti-fungal activity of irradiated sodium alginate treated betel leaves

3.2.10 Shelf life of SA treated betel leaves

After 30 days foliar spraying of various concentration of irradiated SA on betel vines, matured leaves were collected and weighed. The wet weight of 350 ppm SA treated leaves the best weight gain (59g) than control (22g). After weight taken, these leaves were preserved separately in an airtight bag and then the treated and untreated both samples were stored in a refrigerator at 8°C. Weight gained in 350 ppm treated betel leaves 168% greater than control. Effect on damaged percentage and weight gain in SA treated betel leavespresented in table 7. After one month, it was observed that only 350 ppm SA treated leaves extend their shelf life of 50 % seeing as 30 days which shown in figure 5.

These results are consistent with other scientists in case of mango, carrot, and tomato. The crab chitosan irradiated (200 kGy) had extended the shelf life of mango fruits. Another study showed that chitosan extends the shelf life of litchi. Chitosan coating was seen to delay fruit senescence of strawberry fruits stored at 10ºC and 70 ± 5% relative humidity.

Table 7: Effect on damaged percentage and weight gain in SA treated betel leaves.

Sample ID	Damaged (%)	Total weight (in g)
Control	100%	22.3
350 ppm	50%	59.0
500ppm	100%	44.5
750ppm	100%	39.2

Figure 5. Photograph of the betel leaves after 30 days kept in a refrizerator.

3.2.11 Sensory evaluation test

A sensory evaluation test was conducted by 70 persons by eating treated and untreated betel leaves. Except this, by a survey it distinguished that most of the peoples didn't know why they chew this leaves. The leaves obtained from the sample treated at 500 ppm were passed the test scoring ±8 out of 10, where the control sample in acceptance of 3 represents in table 8. Betel leaves contain alkaloid [18] and phenolic compounds which might be the causes of taste variation.

Table 8: The sensory evaluation test of irradiated SA treated piper betel leaves (by Ranking Procedures)

Person	**Specimen**	**Color**	**Texture**	**Appearance**	**Taste**
70	Control	4± 0.23	3 ± 0.16	3 ± 0.05	3 ± 0.08
	NSA	4 ± 0.22	5 ± 0.22	5 ± 0.23	5 ±0.11
	350 ppm	7± 0.12	7±0.12	7 ± 0.46	6± 0.54
	500 ppm	8 ± 0.4	7 ± 0.34	7 ± 0.26	8 ± 0.66
	750 ppm	6 ± 0.11	5 ± 0.11	6 ± 0.11	7 ± 0.56

3.2.12 Scanning electron microscopy of pure and irradiated SA treated betel leaves

The SEM images of the control (Figure 6a) and irradiated SA (Figure 6 b, c, d) are shown in Figure 6. The SEM image of irradiated SA and control is almost same.

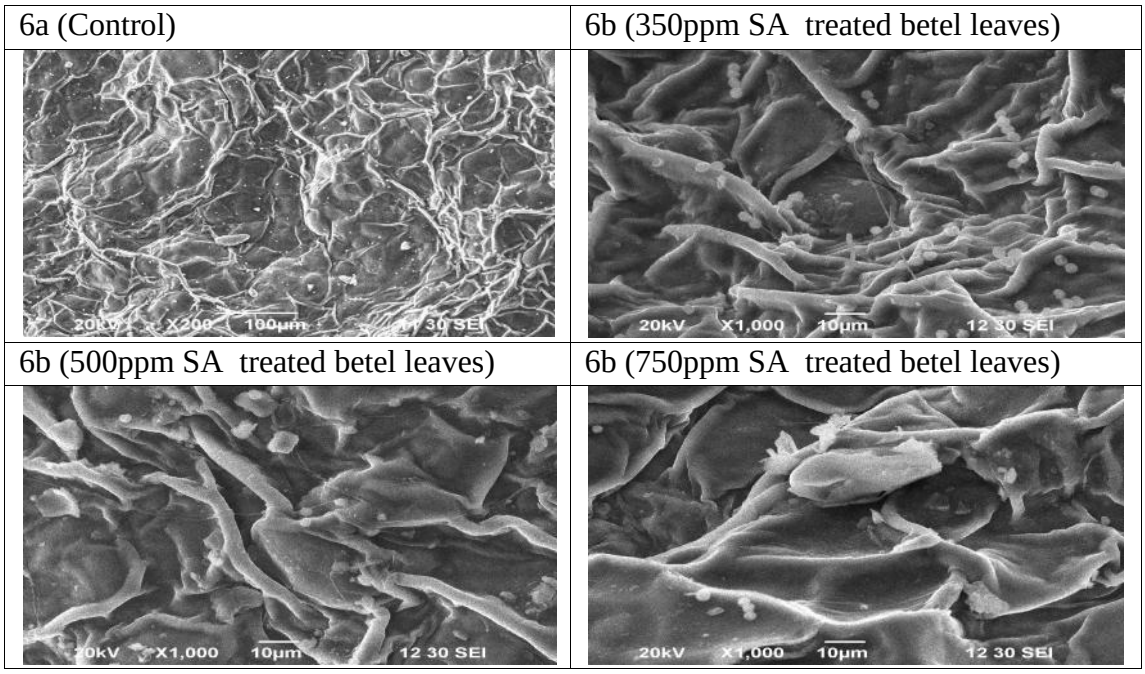

Figure 6. SEM photograph of the SA treated betel leaves

3.2.13 TG of SA treated betel leaves

Table 9 represents the weight loss percentage of control and different concentration of 12 kGy irradiated SA treated belel leaves. The changes in weight loss of irradiated SA are not significant at different doses applied. However the weight retain percentage of irradiated SA and control is almost same. The result showed that alginate was not depointed on the betel leaf; rather they have been significantly absorbed by the betel vine as noticeable change has been obsered in nutrient level.

Table 9: Weight retain percentage of SA trated betel leaves

Sample ID	Weight retain percentage at various temperatures (°C)						
	0-25°C	100-110°C	200-250°C	300-350°C	400-450°C	500-550°C	550-600°C
Control	100%	94%	89%	66%	52%	24%	20%
350 ppm	100%	94%	86%	63%	51%	25%	20%
500 ppm	100%	94%	85%	66%	52%	25%	20%
750 ppm	100%	94%	85%	66%	52%	25%	20%

3.2.14 Observations

- After 30 days foliar spray of NSA and irradiated SA on betel vine plants 500 ppm SA treated betel vine showed the best plant height of 72%.
- Control plants achieved matured number of leaves 22 whereas NSA, 350 ppm, 500 ppm and 750 ppm treated betel vines achieved 24,26,43,30 leaves respectively than control. Betel vine treated with 500 ppm SA showed the best leaves production than control.
- Betel vine treated with 750 ppm SA showed the best leaves area of 100%. Shoot growth increased the best at 350 ppm SA treated betel vines of 250% than control.
- The best effects on root length and thickness were increased at 31%, 49% for 500 ppm SA treated betel vine as compared to the control plants.
- There have found an increase of 288% in the fresh weight of betel leaves with compared to control.
- After 30 days foliar spraying of 750 ppm SA on betel vines, Fe and Ca increases 110 % and 31% respectively than control leaves.
- In 350 ppm SA treated betel leaves, Pb and Cr consumption decreases 96% and 120% respectively than control.
- 350 ppm irradiated SA is causing complete inhibition of disease control almost 100% after 30 days foliar spraying of irradiated SA on betel vine plants than control.
- With the SA treatment of 350ppm concentration on betel vine plants, a total fungal count was decreased.
- 350 ppm SA treated leaves extend the best shelf life of 30 % as compared to other concentrations, and weight gained 168% than control seeing as 30 days.
- The weight retain (%) of irradiated SA treated betel leaves and control was found on more or less same; there was no significant change was observed for TG analysis. The changes in SEM image of irradiated SA treated betel leaves and control leaves are also alike.
- Betel vine showed increases of plant height, number of leaves, leaves area, number of shoots, root length & thickness, number of stems from 25 to 288 % in different cases after the 30 days application of irradiated SA on betel vine. The growth promotion activity of the irradiated SA varies on different parts of the plants at a different rate. A significant amount of Fe and Ca uptake was observed on betel vine after the alginate application. The chelating capacity of alginate increases uptake of some plant nutrients from the soil. The leaves obtained from the sample treated at 500 ppm irradiated SA were passed the test scoring ±8 out of 10, where as the control sample got in

acceptance of 3. After 12 kGy radiation dose, the molecular weight of SA was achieved around 5000Da. This study suggests that gamma irradiated alginate can be used as plant growth promoter.

3.2.15 References

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Chapter 4: Results and Discussion

(Section 4.1: Application of irradiated CS on selective crops)

4. Results & Discussion

Section 4.1: Application of irradiated CS on selective crops

4.1.1 Introduction

Radiation processed CS even at very low concentrations of a few tens of ppm is very effective for use as plant growth promoter. This application offers tremendous opportunity to use it as highly effective organic fertilizers. Besides, the biodegradability of natural macromolecules will be an additional advantage of using such materials as plant growth promoters [1-3]. The antibacterial and antifungal effect of low molecular weight CS has been demonstrated for reducing the post-harvest losses by prolonging the shelf life of many fruits and vegetables by coating them with these polysaccharides. More importantly, low molecular weight CS has been demonstrated to be an effective plant protector against infectious diseases and environmental stress [4].Among various techniques used for the modification of polymer properties, the use of ionizing radiation either in photonic (gamma radiation, X rays) or particulate forms (accelerated electrons, ion beams) has proven to be a very convenient technique. Molecular weight and its control is of very important for its particular end-use.radiation-induced crosslinking has long been the main reason for the use of high energy radiations in polymer processing. The opposite effect of radiation, in other words, chain scissoring or degradation of polymers has not found great interest until recently. The degradation effects of ionizing radiation have been generally connected with the chemical structure of polymer chains, presence or absence of some additives and irradiation atmosphere, the presence of oxygen or air leading mostly to radiation-induced oxidation. Polymers carrying quaternary carbon atoms in the main chain suffer from chain scission. Most of the natural polymers carrying oxygen atom in their main chains also degrade upon irradiation. CS, cellulose and other polysaccharides are examples of repeating -C=O- groups on their backbones undergoing main chain scissoring. A different interpretation of structure vs. chain scissoring sensitivity is based on the heat of polymerization of monomers. Polymers showing relatively low heats of polymerization tend mostly to degrade upon irradiation. CS undergoes predominantly chain scission when exposed to ionizing radiation which results in the production of low molecular weight products named oligomers or oligosaccharides. These oligomers are easy for uptake by the plant. Radiation has also shown no effect on the functional groups (NH_2) of CS (figure 1) but instead enhance the anti-microbial activity of the oligosaccharides that improved the plant resistivity towards fungi attacked. These oligomers have potential application in agriculture as plant growth promoter [5].

CS follows chain scission, cross linking or radical formation with the use of ionizing radiation [6]. All the C-H sites in the pyranose ring as well as the substituent CH_2OH group are involved in the free radical forming process with very poor selectivity toward reactive radicals (like O-H radicals). The amine group is expected to play a

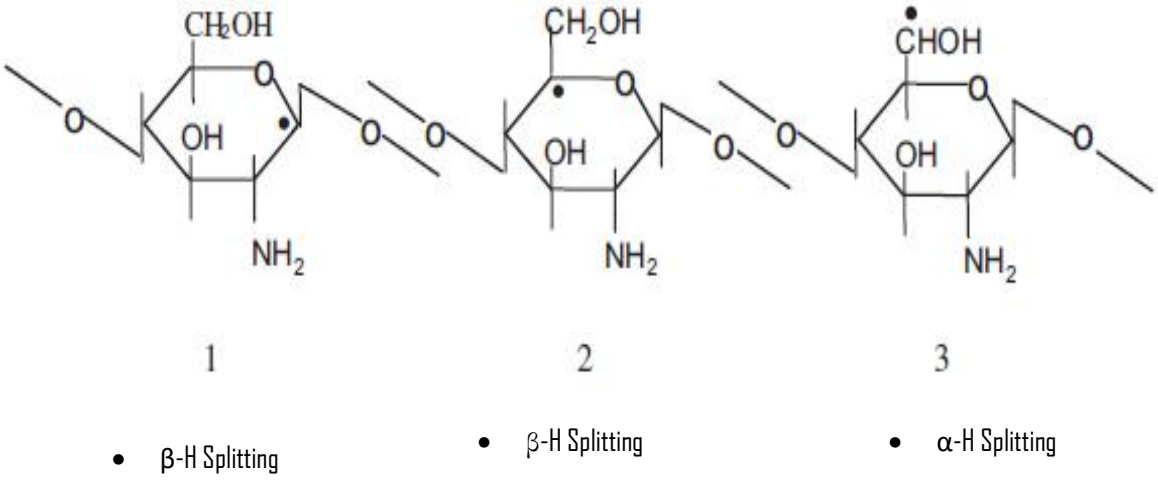

Figure 1: Chain scission mechanism of CS when expose to ionizing radiation which produces low molecular weight CS[6].

prominent role as compared to the O-H groups due to its lower ionization potential. Electron loss from the nitrogen lone pair would prompt the formation of aminyl and alkyl amino radicals. Ketones, aldehydes and carboxyl units in the structure of irradiated carbohydrates are formed mainly as a consequence of the thermal instability of the radicals with free valencies at the C_1,C_4, C_5 positions in the pyranose ring.These species undergo β-scission below room temperature with ring opening (radical C_5) giving secondary reactive radicles and carbonyl bearing activated C-bonds. The subsequent H-abstraction reactions favored by the close proximity of the reactive centers will cause the formation of the stable α-carbonyl end radical products when applied to the CS radiolysis [6].

4.1.2 Molecular weight determination of the CS samples

The figure 2 shows that with the increasing radiation dose, the molecular weight of CS decreases. The increase of radiation dose until 50 kGy significantly decreased the MW of CS.

As compared to the conventional techniques, like acid or base hydrolysis or enzymatic methods, radiation processing offers a clean one-step method for the formation of low molecular weight polysaccharides [7].

The molecular weight at 50 kGy is under 1000000 which can be used as a good plant growth promoter. The decreasing molecular weight suggests that the dose of radiation causes the chains of the polysaccharide groups to shorten by the scission of glycosidic bonds.

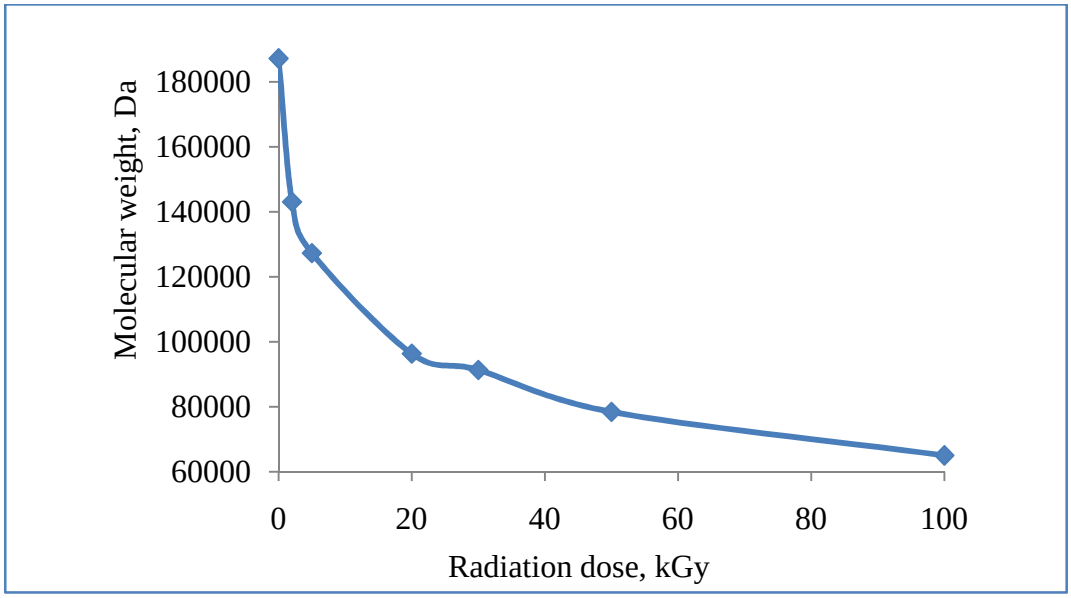

Figure 2. Effect of gamma radiation (0-100 kGy) on molecular weight of CS

4.1.3 Characterization of chitin and CS samples by FTIR spectroscopy

FTIR spectroscopy was used to characterize the chemical functional groups of as synthesized CS. The FTIR spectrums of CS (figure 3) show that the characteristic peak of –OH and overlapping N-H group of 3453 cm^{-1} is quite different. This figure shows that the stretching peak of O-H overlapping N-H group at 3453 cm^{-1} is less for the radiated CS spectra in figure3(a, b and c). Radiation causes chain scission and thus the intensity of peaks for the amine group decreases.From the spectra, it was observed that the significant peaks positions have not changed due to variation in concentration of CS after irradiation. The concentration was in ppm level, this is why, no change was observed at peak position.

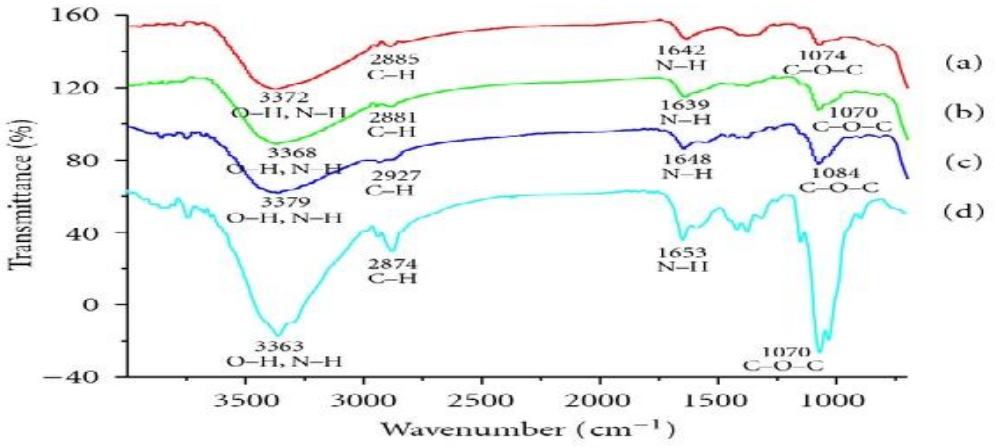

Figure 3. FTIR spectra of NCS (d) and different concentration (a=350 ppm, b=500 ppm, c=750 ppm) of irradiated (50 kGy) CS

4.1.4 Effect of radiation on degree of deacetylation (DD) of CS

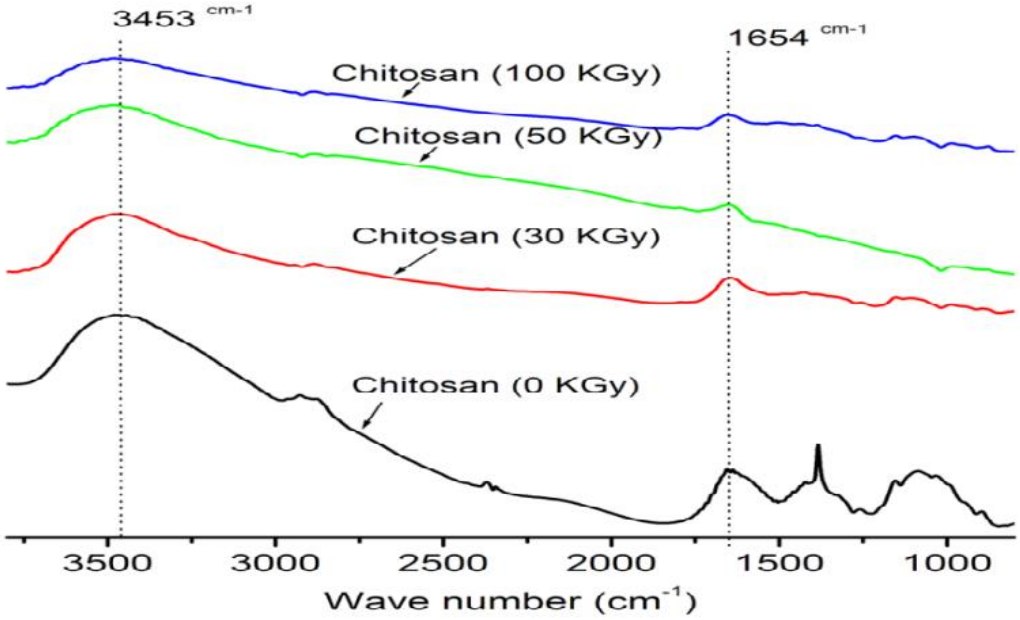

Figure 4. Degree of deacetylation (DD) determination from irradiated (30 kGy, 50 kGy, 100kGy) and nonirradiated (0 kGy) CS samples

DD% was calculated as described in methodology section.

Where A_{1655} and A_{3450} were the absorbance at 1655 cm $^{-1}$ of the amide-I band as a measure of the N-acetyl group content and 3450 cm $^{-1}$ of the hydroxyl band.This figure shows that DD of CS did not vary largely due to different radiation dose. It was obtained in a range of (74-80) %, shown below in table 1.

Table 1: Effect of radiation on degree of deacetylation (DD) of CS

Radiation Dose (KGy)	Absorbance at 1655 cm^{-1}	Absorbance at 3450 cm^{-1}	% Degree of deacetylation
0	0.0614	0.2696	74
30	0.0294	0.1603	78
50	0.0215	0.1204	79
100	0.0215	0.1235	80

Considering the above results from table 1, it can be said that the process of CS irradiation at reported doses did not affect the DD, since no significant differences

were found among the samples. Researcher [8] described the trend for irradiated CS using different doses. They reported that the scission mechanism of CS is based only on the scission of glycosidic bonds of CS molecules by gamma irradiation and the remaining functional groups of the polymer are not affected by the attack of free radicals. As in the present research, Zainol et al. (2009) [8] also reported no significant changes in the values of DD that ranged between 71 and 74% vs. irradiation doses of 0, 10, 25, 50 and 100 kGy. Meanwhile, Lim et al. (1998) [9], who worked with highly deacetylated CS (98.17%) irradiated until 25 kGy, also obtained no significant difference in the DD (98.08–98.93%) before and after irradiation.

Unlike these authors, others suggest that the DD increases with the irradiation dose. In this case, Khan (2012)[8] determined the DD of CSs irradiated at 0, 2, 5, 20, 30, 50 and 100 kGy and reported values of 73, 74, 77, 78, 78, 79 and 79% for the DD, respectively. This behavior was related with a decrease in transmittance as observed with increasing irradiation dose[11]. This fact is associated with the hydrolysis of acetamide to amine, favored by ionizing radiation[12].

Contrary to these results, Shen et al. (2011)[13] reported that the DD decreased when the irradiation dose increased from 0 kGy to 10, 20, 50, 100, 150 and 200 kGy, respectively. Another researcher (U.Gryczka et.al.) reported that CSs were irradiated at 0, 10, 20, 50, 100, 150 and 200 kGy, with significant differences in the values of DD, especially against 10 and 20 kGy, for which the value decreased from 88.5 to 84.1%. From 20 to 50 kGy DD decreased again to 70.8%, In this case, the decrease in DD is not the result of an increase in the amide groups, but the decrease of the amino groups (NH_2), due to the effect of chain scission of the polymer by irradiation. It is proposed that, following irradiation some NH_2 groups of CS will be removed and converted to gaseous ammonia after joining with the hydrogen radicals. These results did not agree with those informed by Zainol et al. (2009)[8], who concluded that the macromolecule is cleaved only by the glycosidic bonds, and that the remaining functional groups are affected by the irradiation, but these differences may be due to the state of CS, namely, a gel [13] or powder [8].

4.1.5 Application of CS as a growth promoter on selective crops

Polysaccharides are the most abundant of the four major classes of biomolecules, which also include proteins, lipids and nucleic acids. They are often classified on the basis of the sequences and linkages between their main monosaccharide components, as well as the anomeric configuration of linkages, the ring size (furanose or pyranose), the absolute configuration (DD) and any other substituents present. Certain structural characteristics such as chain conformation and intermolecular associations influence the physicochemical properties of polysaccharides. For example, polysaccharides containing large numbers of hydroxyl groups are often thought of as being hydrophilic. Polysaccharides fill numerous roles in living organisms, such as the storage and transport of energy (e.g. starch and glycogen) and structural components

(e.g. chitin/CS, cellulose). Due to unique biophysical and chemical properties of CS, such as biocompatibility, biodegradability, nontoxicity and nonantigenicity), a broad spectrum of applications has been emerged in different modern fields: water treatment (Kurmaev et al., 2002)[14], chromatography, additives for cosmetics, textile treatment for antimicrobial activity (Hai et al. 2003)[15], novel fibers for textiles, photographic papers, biodegradable films, biomedical devices, and microcapsule implants for controlled release in drug delivery. Tissue engineering and adsorption of metal ions as well as removal of dyes are some of the many applications of CS have been reported (Jayakumara et al., 2005) [16]. 2% w/v, CS solution were prepared and then irradiated at 50kGy. Irradiated CS and non-irradiated CS samples were applied on tomato, spinach, mung dal, jute, pumpkin, egg plant, green chilli, red chilli, cabbage and cauliflower plants for 90 days at 15 days of intervals and their photographs are shown in figure 5 and sample ID for figure 5 represents in table 2. The growth promotion activity of these plants was tested on various growth parameters which are averages of plants height, number of leaves & stems, leaves area, vine's thickness, root length, size of fruits, number of fruits, harvest time, first flowering, wet weight yield. Figure 5 shows the control and CS treated selective crops.

Table 2: Sample ID for figure 5

Control	Treated
a. Egg plant	b. Egg plant
c. Tomato	d. Tomato
e. Pumpkin	f. Pumpkin
g. Spinach	h. Spinach
i. Mung dal	j. Mung dal
k Jute	l. Jute
m. Cabbage	n. Cabbage
o.Cauliflower	p. Cauliflower
q. Green chilli	r. Green chilli
s. Red chilli	t. Red chilli

1.6 Growth promotion effect on plants height

The figure 5 and 6 shows that the changes of plant height after 90 days due to applying nonirradiated and irradiated CS (at 50 kGy) of different concentrations. An enhancement of plant's height of green chilli, red chilli, mung dal have increased due to the application of 100, 100, 200ppm and pumpkin, spinach tomato and jute increased by 300ppm, 500ppm 1000ppm concentration of the CS solution respectively.

Pumpkin and egg plant's height have increased upto the application of 500ppm concentration of the CS solution and with the increase in concentration of the

5 a	5c	5 e
		
5b	5d	5f
		
5g	5i	5k
		
5h	5j	5l
		
5m	5o	5q
		
5n	5p	5r
		
5s	5t	
		

Figure 5. Photographs of CS treated selective crops

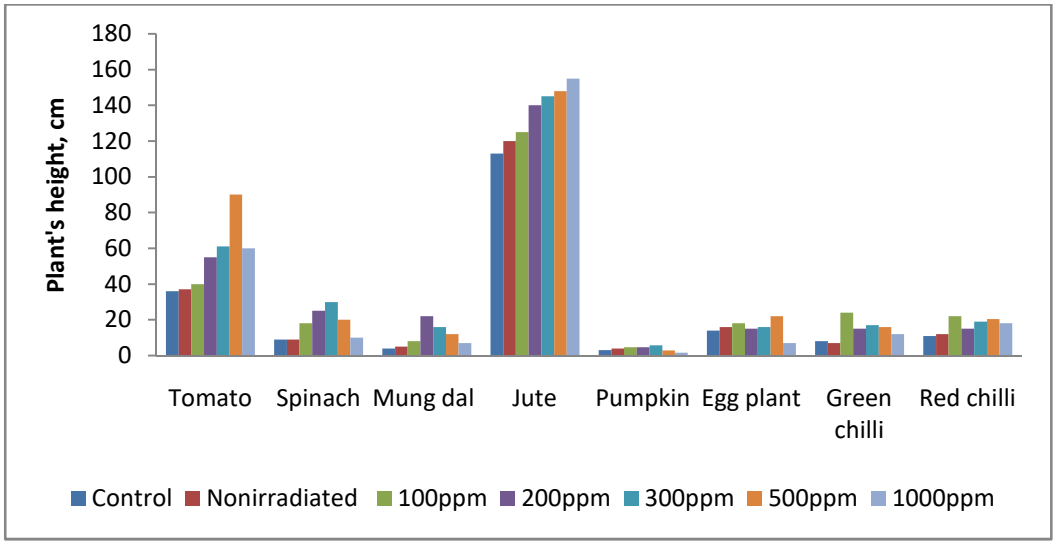

Figure 6. Effect of nonirradiated and irradiated CS on the plant's height of selective crops after 90 days of foliar spray.

solution growth have not increased. It is observed that the lower concentration of the solution penetrated into these plants cell easily and which causes higher chlorophyll content.

Ionizing radiation processed oligosaccharides have various novel biological effects such as plant growth promotion, anti-microbial activity, activation of immune system (activation of macrophages, stimulation of cytokine production, induce of phytoalexin), etc. [1-3].

4.1.7 Growth promotion effect on shoot growth

The figure 7 shows that the changes of shoot growth after 90 days due to applying nonirradiated and irradiated CS (at 50 kGy) of different concentration.

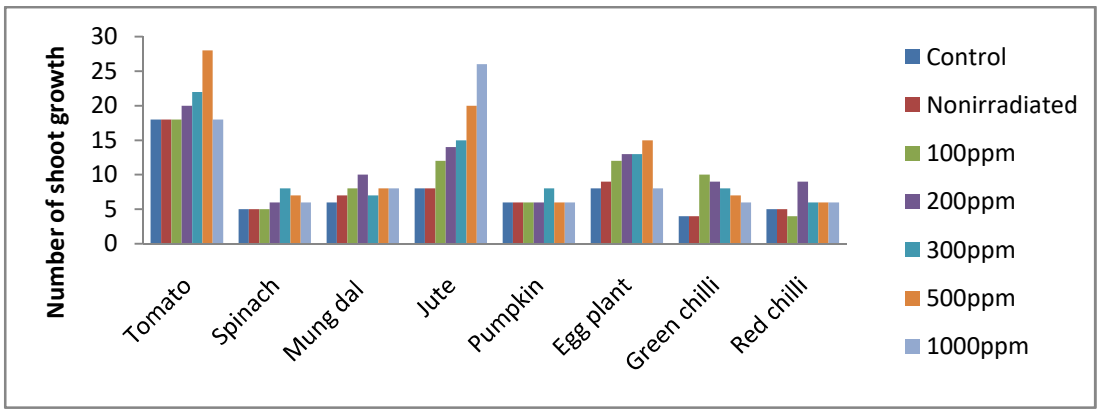

Figure 7. Effect of nonirradiated and irradiated CS on the shoot growth of selective crops' after 90 days of foliar spray.

It was observed that, an enhancement of shoot growth of green chilli, red chilli, mung dal have increased due to the application of 100, 100, 200 ppm and pumpkin, spinach tomato and jute increased by 300 ppm, 500 ppm 1000 ppm concentration of the CS solution respectively. Moreover, the treated samples have shown an increase in shoot growth than those of the control and NCS.

4.1.8 Growth promotion effect on number of leaves

The figure 8 shows that the changes of number of leaves after 90 days due to applying nonirradiated and irradiated CS (at 50 kGy) of different concentration. The counting of leaves of the test plants were started after 15 days of germination to 90 days and at the end of the experiment the data showed that increased leaves were obtained for the specimen treated by 50 kGy irradiated CS, than the control specimen. Leaves of green chilli, red chilli, mung dal have increased due to the application of 100, 100, 200 ppm and pumpkin, spinach tomato and jute increased by 300 ppm, 500 ppm 1000 ppm concentration of the CS solution respectively.

Moreover, the treated samples have shown an increase in number of leaves than those of the control and NCS. This results evident that the increased number of leaves stimulate more photosynthesis and the growth of the each part of the plant is greater for the use of irradiated CS than NCS treated and control specimen.

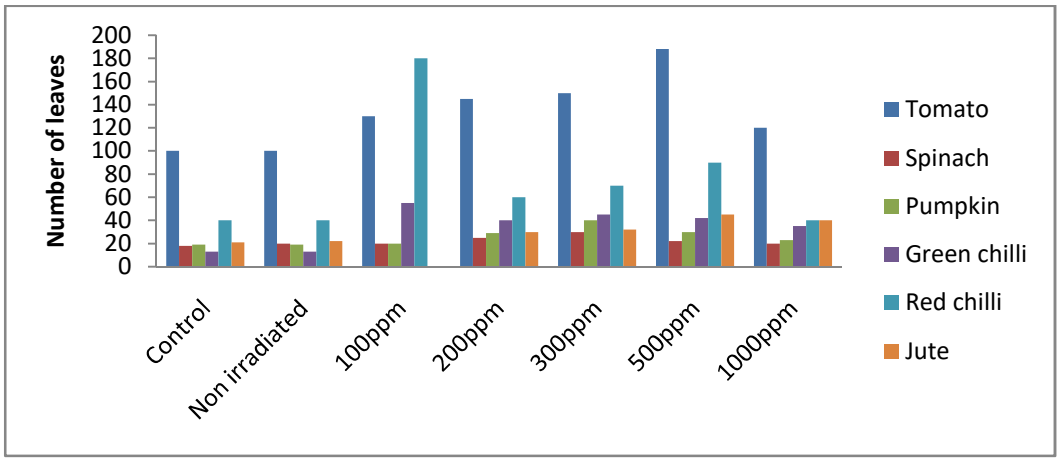

Figure 8. Effect of nonirradiated and irradiated CS on the number of leaves of selective crops' after 90 days of foliar spray.

The increase in total N content in the leaves brought about by the amino components in CS and higher ability of the plant to absorb N from the soil when CS was degraded in soil.

4.1.9 Growth promotion effect on leaves area

The figure 9 shows that the changes of leaves area after 90 days due to applying nonirradiated and irradiated CS (at 50 kGy) of different concentration. The leaf areas

of the plants were measured according to the procedure described in the experimental section. As it is cumulative leaf area, there is no data for the first day of examination. At the last day of experiments, leaves area were measured.The graph shows that the enhancements of the leaf area of the treated specimen were greater than the untreated specimen for all plant specimens.

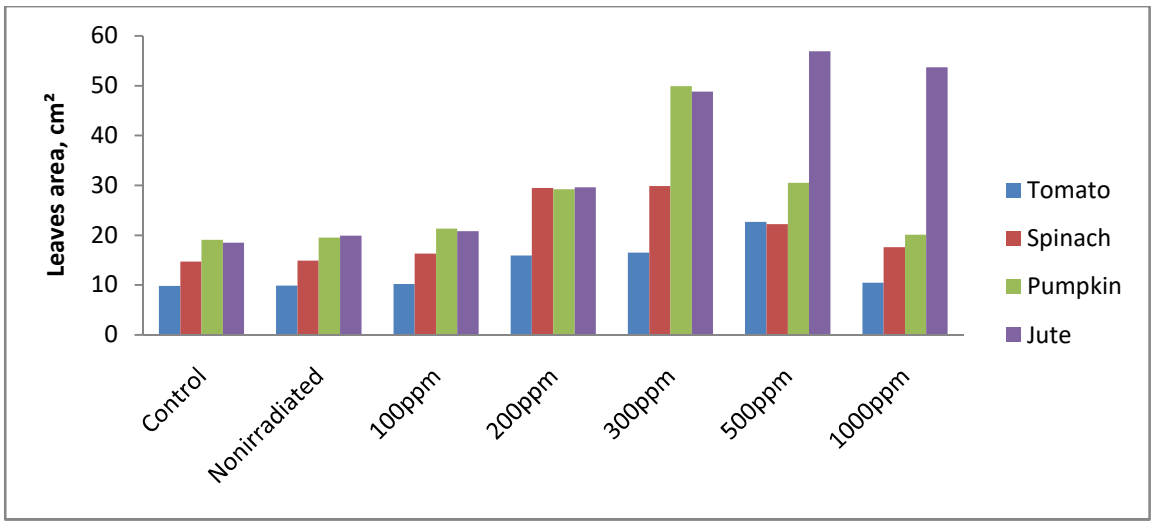

Figure 9. Effect of nonirradiated and irradiated CS on the leaves area of selective crops' after 90 days of foliar spray.

4.1.10 Growth promotion effect on production of fruits

The figure 10 shows that the effect on production of fruits after 90 days due to applying nonirradiated and irradiated CS (at 50 kGy) of different concentration. The graph shows that the increased fruits productions of the treated specimen were greater than the untreated specimen for all plant specimens.

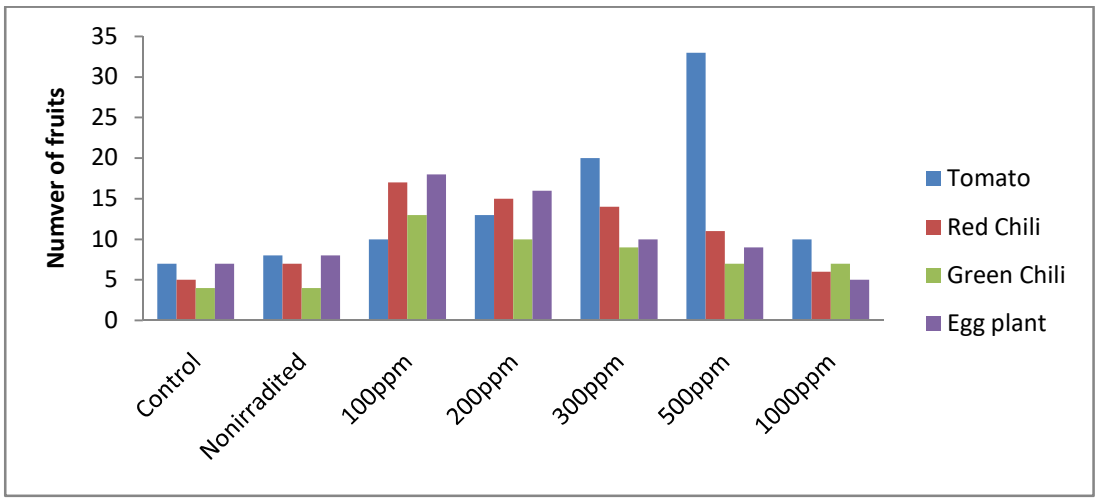

Figure 10. Effect of nonirradiated and irradiated CS on the number of fruits of selective crops' after 90 days of foliar spray.

Recently, it has been documented that these oligomers are applied to plants in the form of hydroponics method or by foliar sprays, they elicit various kinds of physiological activities, including plant growth in general, seed germination, shoot elongation [3, 17-19], root growth [20], flower production, antimicrobial activity, amelioration of heavy metal stress, phytoalexin induction, etc. [21].This also clear in the higher content of sugar contains in the fruits.

4.1.11 Growth promotion effect on size of fruits

Figure 11 & 12 showed the growth promotion effect on sizes of fruits (diameter of fruits in cm^2) of different plants after 90 days due to applying of nonirradiated and irradiated CS of 100 to 1000 ppm concentration on 90 days of foliar spray. It has also noticed that the sizes of the fruits were also increased after the treatment applied. Figure 12 showed the photograph of control and irradiated CS applied cabbage (figure12a,12b), cauliflower (figure 12c, 12d) plants along with their fruits (table 3).

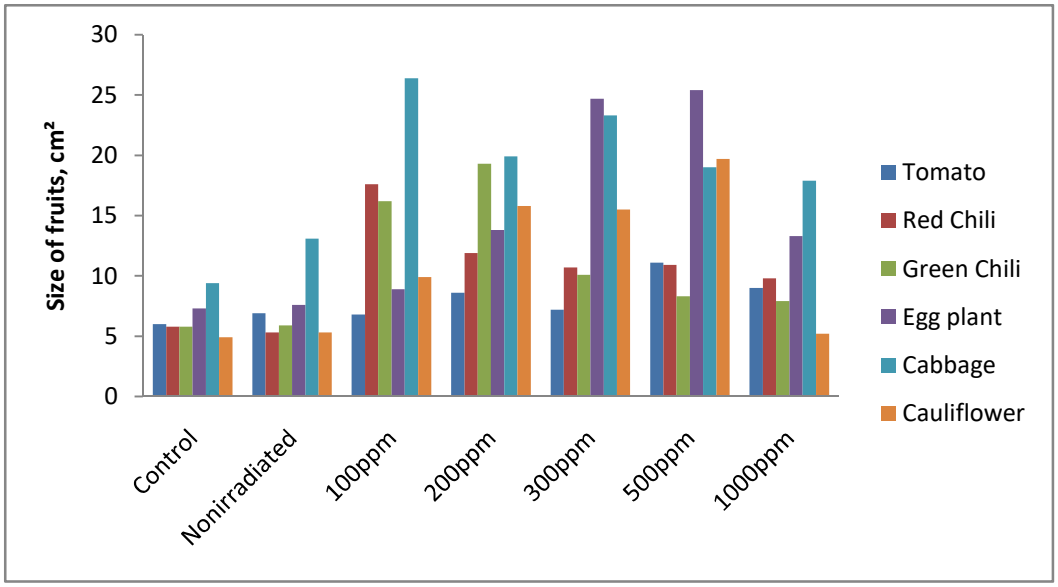

Figure 11. Effect of nonirradiated and irradiated CS on the size of fruits of selective crops' after 90 days of foliar spray.

Bio-stimulators are a category of products which, by definition, increase plant productivity especially under unfavorable conditions through an increase of the plant's ability to cope with stresses. Despite that the improvements are usually not very spectacular, often statistically insignificant, and not always stable over the years; farmers' interest on using them is increasing yearly [22]. A lot of studies have been carried out to investigate the plant growth promotion and plant protection effect of radiation processed polysaccharides in a variety of crops under different environmental conditions. The results have clearly shown that radiation processed polysaccharides even at very low concentrations are very effective for use as organic fertilizers. Alginate, degraded with a radiation dose up to 500 kGy under electron

12(a)	12(b)
	
12(c)	12(d)
	

Figure 12. Photograph of nonirradiated and irradiated CS treated cabbage and cauliflower size after 90 days of foliar spray.

Table 3: Sample ID for figure 12

a. Cabbage (control)	b. Cabbage (100 ppm treated)
c. Cauliflower (control)	d. Cauliflower (500 ppm treated)

beam promotes the growth of rice seedlings. The foliar spraying of degraded alginate on tea, carrot and cabbage led to an increase of their productivity by 15–40% [3]. Degraded polysaccharides such as alginate, CS or carrageenan, in concentrations from 20 to 100 mg/ml, increase tea, carrot or cabbage productivity by 15–40%.

4.1.12 Growth promotion effect on root length

The figure 13 shows that the increased root length of selective plants' after 90 days due to applying of NCS and different concentration of 50 kGy irradiated chotosan. It was observed that increased root length of tomato were found at 500 ppm solution concentration and for mungdal, pumpkin and red chilli plant shows increased root length after applying 200 ppm solutionthan control. 100ppm solution concentration

showed the best effectiveness on root length of spinach, 300 ppm for egg plant and green chilli and increased root length of jute were found at 1000 ppm solution concentration. Figure 14 showed the photograph of control and irradiated CS applied jute (figure14a, 14b), green chilli (figure14c, 14d), red chilli (figure14e, 14f) plants along with their roots (table 4). It was observed by figure 14 the root of tomato plants increased upto 32.6 cm due to applying 500 ppm irradiated CS than that of control (10.9 cm). Thus almost 292% increased root indicated the remarkable growth of tomato plants.

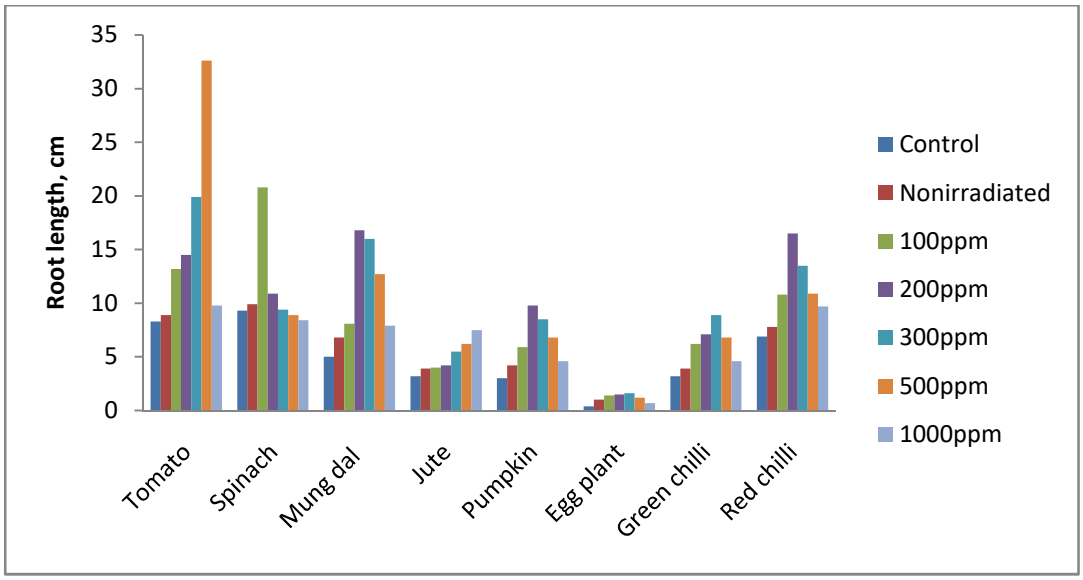

Figure 13. Effect of nonirradiated and irradiated CS on the root length of selective crops' after 90 days of foliar spray.

A positive effect of CS was observed on the growth of roots, shoots and leaves of various plants including Gerbera [23] and of several crop plants [24]. However, there is no significant change observed due to concentration. Although, chlorophyll was not measured in this study. Chbu and Shiayama (2001) [24] reported higher chlorophyll content in the CS treated plants.

Table 4: Sample ID for figure 14

14a. Jute (control)	14b. Jute (ppm treated)
14c. Green chilli (control)	14d. Green chilli (300 ppm treated)
14e. Red chilli (control)	14f. Red chilli (200 ppm treated)
14g. Tomato (control)	14 h. Tomato (500 ppm treated)

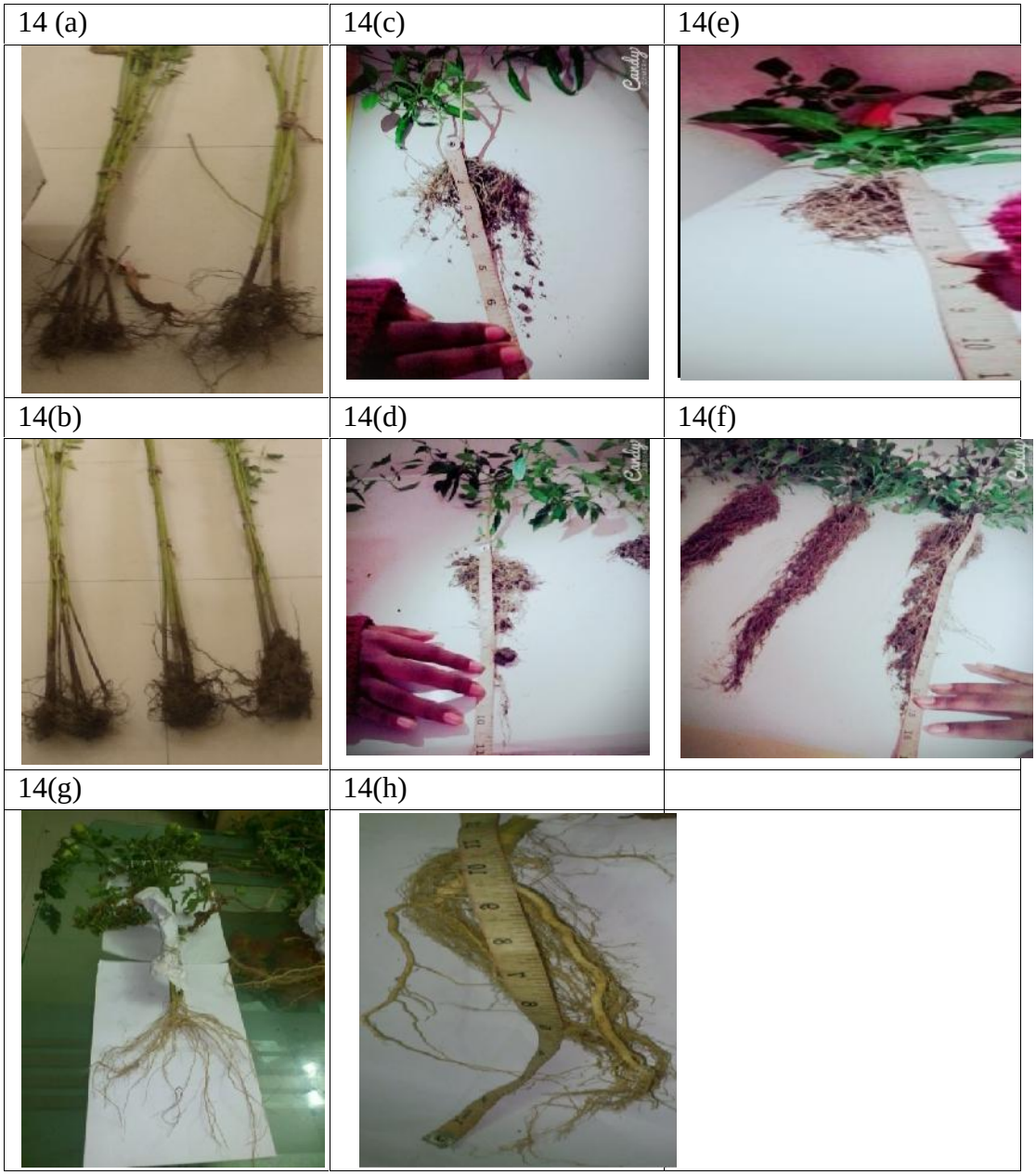

Figure 14. Photograph of the nonirradiated and irradiated CS treated crops' root length on 90 days of foliar spray.

Both factors (higher number of leaves and chlorophyll content) contribute to a more photosynthesate production which reflects on higher dry weight and production of the plants and this is what has been observed in this study. The increase in dry weight reflected on the yield components namely leaves number, stem number and stem size.

4.1.13 Growth promotion effect on reduction of disease

The figure 15 shows that the effect on reduction of disease of selective plants' after 90 days due to applying of nonirradiated and at different concentration of 50 kGy irradiated chotosan. To detect the disease control; the affected leaves were counted

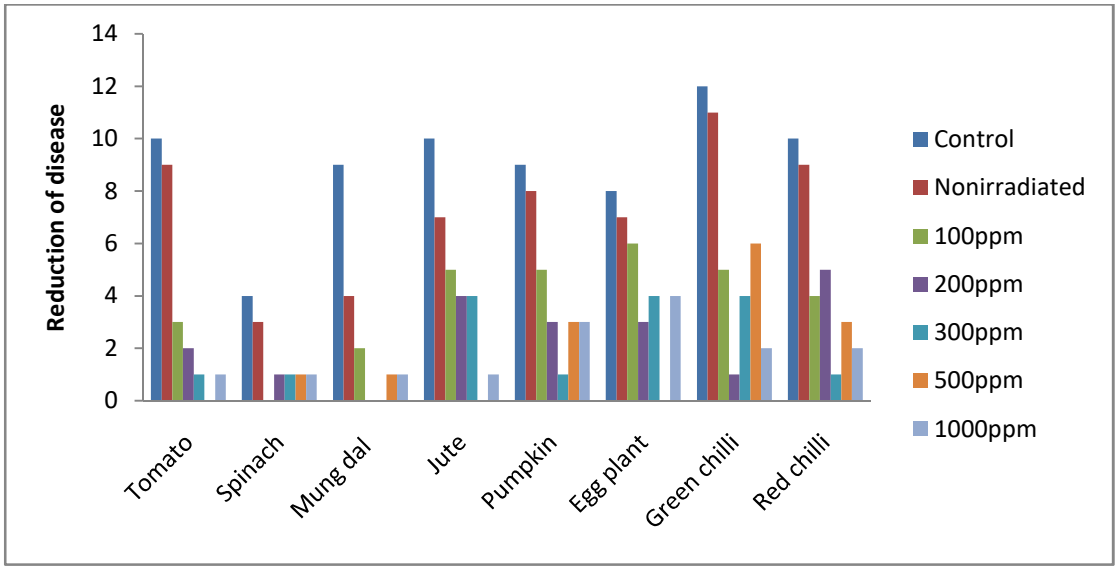

Figure 15. Effect of nonirradiated and irradiated CS on reduction of disease of selective crops' after 90 days of foliar spray.

after every 7 days. The numbers of affected leaves were observed more in control sample than treated sample. Oligo-CS act as a biotic elicitor to enhance defense responses against diseases. It has been recognized as potent phyto-alexin inducer to resist infection of disease for plants.

4.1.14 Observations

- After applying 50 kGy radiation dose, molecular weight of chitosan was achieved 90000 Da and DD% was achieved 78%.
- After 90 days of foliar spray of NCS and irradiated CS on selective crops the highest plant height was observed in mung dal and egg plant at around 340% than that of control.
- The best shoot growth was observed for jute plant at 225% over the control one.
- The number of leaves was found increased highest on red chilli.
- The leaves area was increased 207% for jute among all the crops.
- The productivity of tomato showed 371% more than that of control.
- The size of fruits increased 302% in case of cauliflower and it is the highest growth in fruit size among all the crops.
- The highest root length was achieved in tomato around 292% than that of control.
- Green chilli plants showed no disease after 90 days foliar spray of irradiated CS.
- Significant reduction of diseases was observed on those crops after the irradiated CS application.

4.1.15 References

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Section 4.2: Application of irradiated CS on betel vine

4. Results & Discussion

Section 4.2: Application of irradiated CS on betel vine

4.2.1 : Introduction

Betel leaf is an export item and one of the cash crops of Bangladesh. Betel vine (Piper betel L.) is a perennial dioeciously creeper belonging to the family Piperaceae. Its cultivation is concentrated in the greater district of Barisal, Cox's Bazar, Rajshahi, Maulavi- Bazar, Satkhira, Jessore, Kushtia, Jhinidah, Pabna etc. The acreage of betel vine is decreasing gradually because of some physical and socioeconomic barriers like unavailability of credit facilities, uncontrolled marketing system and infestation of diseases and pest. The deep green heart-shaped leaves of betel vine are popularly known as Paan in Bangladesh. Some researcher reported on a comparative study of some soil properties in forested and deforested areas in Cox's Bazar and Rangamati Districts, Bangladesh [1] and investigation on foot and root rot of betel vine (Piper betle L.) in Kushtia district of Bangladesh was studied by Jahana et.al., 2016 [2]. It was revealed that Betel vine crop is mainly attacked by foot and root rot disease in Kushtia. Young stems were found more prone to attack than the old ones. Disease incidence and severity of foot and root rot of betel vine ranged from 24.00 to 58.00% and 17.65 to 34.75%, respectively. Considering all the locations of Kushtia District, the maximum disease was recorded in the month of July and the minimum was in October.

Betel leaves was an export item of Bangladesh. In the meantime, the European Union bans our export for Salmonella detection in betel leaves. For identify and solve this problem and to carry out a study with irradiated CS, this research was to elaborate the method of γ-irradiation of chitosan to get different oligomers and studying the effect of such oligomers on the growth promotion and productivity of betel vine.

Like another experiment, various concentration (350, 500 & 750 ppm) of 2% CS solution (which was irradiated by 50KGy) were applied on foliar spraying on betel vine at 7 days of intervals for 30 days. The experimental plot areas were separated for each solution. Plots were prepared by adding natural fertilizer (cow dung) in soil.10 plants were taken for each concentration of these solutions. Thus 50 plants were taken for this research. The solutions of different doses were applied at the rate of 500 ml-per unit plot area of betel vine plantation by using normal hand sprayer. Foliar applications of biomaterial CS were done at every 7 days interval.

After 30 days of treatment, it was observed that 350 ppm solution concentration showed the best growth promotion behavior. Actually, CS plays a vital role in plant growth and act as a bio-fertilizer.

4.2. 2 Growth promotion effect on plants height

Figure 1 shows that the changes of plant height of betel vine plants after 30 days due to applying nonirradiated and irradiated CS of different concentration. Irradiated and non-irradiated CS samples were blended in distilled water (2% w/w) and those dispersions were applied on Piper betel vine. After three months of plantation, the specimen plants of piper betel vine treated with irradiated CS, NCS and without CS (control) are shown in figure 1. The figure indicated that the test specimens treated with irradiated CS had greater growth rate in the view of plant height. Plant heights were monitored after every 15 days intervals are also presented in figure 18. The graph is developed on the day basis, and gave an indication how the plants did increase their height day by day. It also gives an idea that irradiated CS treated specimens yield significantly higher plant height than control and NCS treated specimens. An enhancement of plant height of betel vine was observed at 350 ppm solution concentration. Figure1 proves the plant height on different day of a particular specimen in a cluster.

In the last day of the experiment the growth based on height were measured and 50 kGy irradiated CS sprayed specimens yield a growth about 67% to that of the control one (obtained from NCS).

The treated specimen had shown a greater growth rate over the control one. The lower concentration showed a better result as plant growth promoter.Positive effect of CS was incorporated into the soil and that affect the height of the plants [3].

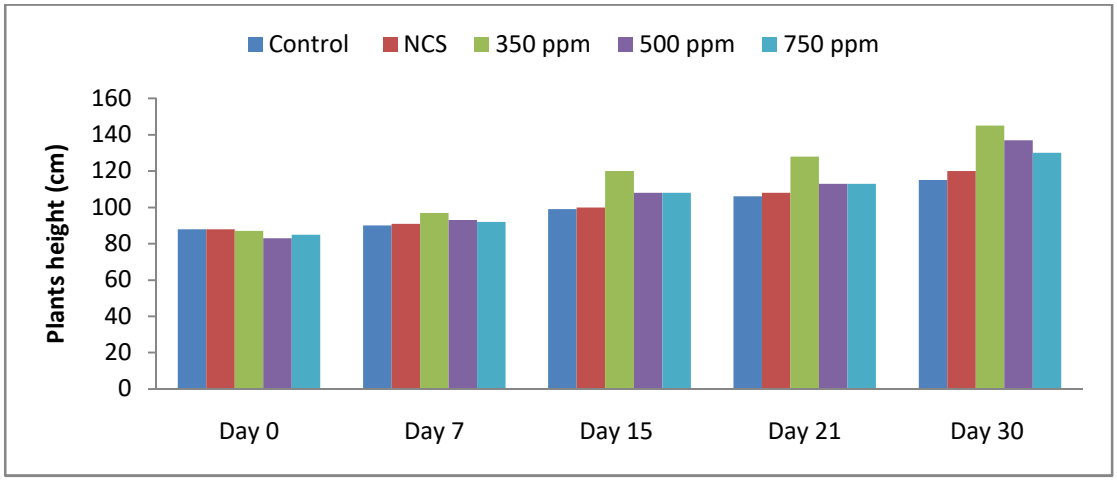


Figure 1. Effect of nonirradiated and irradiated CS on betel vines' height after 30 days of foliar spray.

Barka et al. (2004), showed that the average O_2 production of plantlets cultured on medium supplemented with 1.75% chitogel increased 2-fold, whereas CO_2 fixation increased only 1.5- fold, indicating that chitogel had a beneficial effect on net photosynthesis in plantlets and confirmed its positive effects on grapevine physiology.

It has also been shown that CS promotes vegetative growth and enhances various processes in developing flower buds including induction of flowering of lisianthus (Eustomagrandiflorum) [4].

In the case of irradiated CS the effect on all parameters was positive. When compare its effect to that exerted by nonirradiated form, in respective concentrations, the positive effect was, in all parameters, more evident. In all cases, irradiated and non-irradiated had a stimulatory effect on leaf area, length of roots and newly developed shoots, fresh and dry weights leaf area, than controlled one. The results clearly demonstrated that CS effectively stimulates the developing of roots and shoots of plants.

4.2.3 Growth promotion effect on plants' number of leaves

Figure 2 shows the increased number of leaves after 30 days due to applying of nonirradiated and irradiated CS of different concentration. It was observed that, 500ppm irradiated CS solution concentration showed the best efficiency on productivity of betel vine.

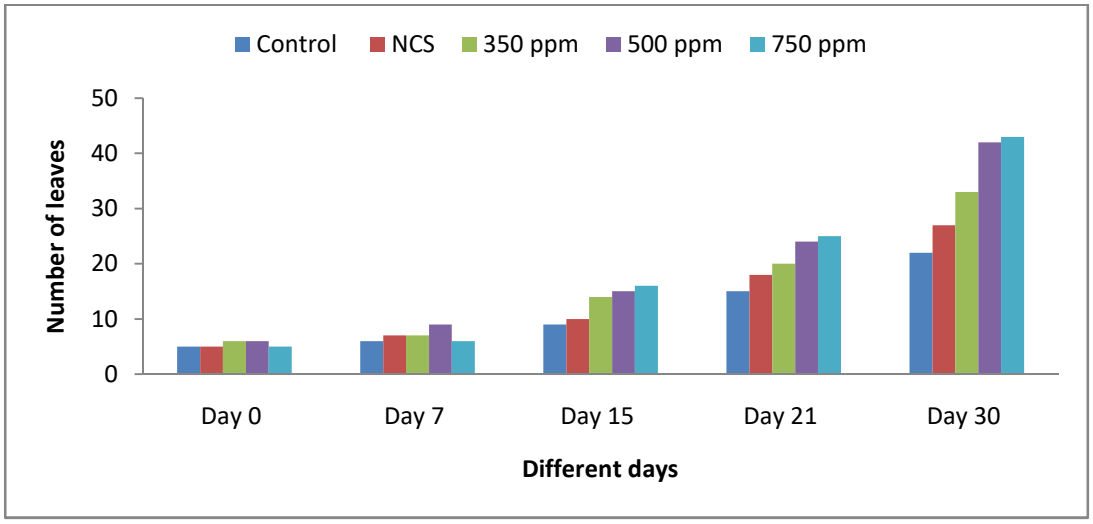


Figure 2. Effect of nonirradiated and irradiated CS on betel vines' number of leaves after 30 days of foliar spray.

Treated specimen had a greater productivity than that of control specimen and the degree of responses differed according to the applied concentration of CS. Number of leaves were observed and found increased with the number of days. The increase in total N content in the leaves brought about by the amino components in CS or higher ability of the plant to absorb N from the soil when CS was degraded helped in plant growth. This results is an evident that the increased number of leaves stimulate more photosynthesis and the growth of the each part of the plant is greater for the use of irradiated CS than NCS treated and control specimen.

4.2.4 Growth promotion effect on shoot growth

Figure 3 shows the increased shoot growth after 30 days due to applying nonirradiated and irradiated CS of different concentration. It was also observed that treated specimen had a greater shoot growth than that of control specimen. However, there is no significant change observed due to concentration.

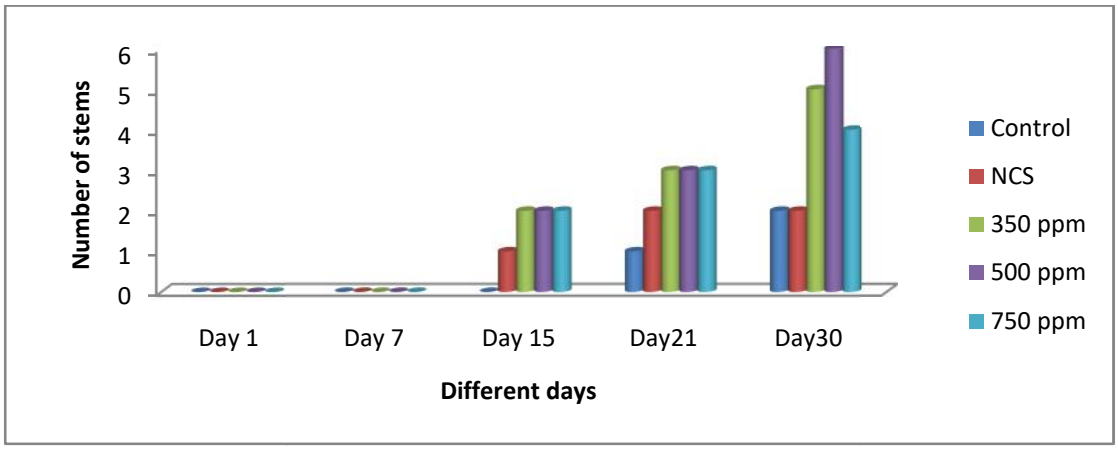


Figure 3. Effect of nonirradiated and irradiated CS on betel vines' shoot growth after 30 days of foliar spray.

Higher chlorophyll content in the CS treated plants reflected on the yield components namely leaves number, stem number and stem size [3]. CS oligomers applied at a concentration of 15 mg L^{-1} stimulate orchid plant growth (Nge et al., 2006). The plants supplied with CS had better developed roots and shoots in the field of agriculture [5].

4.2.5 Growth promotion effect on leaves area

Figure 4 shows the increased leaves area after 30 days due to applying nonirradiated and irradiated CS of different concentration.

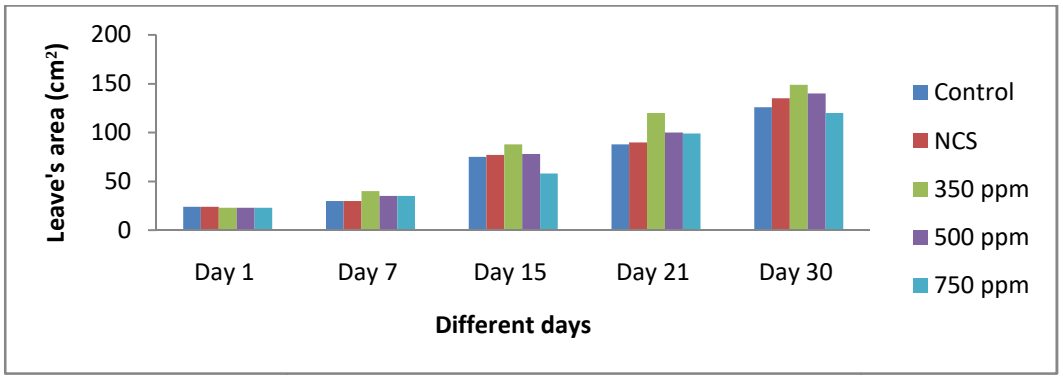


Figure 4. Effect of nonirradiated and irradiated CS on betel leaves' area after 30 days of foliar spray.

At the last day of experiments, leaves area were measured. The figure 4 shows that the CS enhances the vegetative growth in terms of leaves' area were greater than the control specimen. At 350 ppm leaf's area has increased highest (78%).

CS when irradiated at a dose rate of 70–150 kGy strongly affected the growth of rice and wheat plants and reduces the damage caused by vanadium. Irradiated CS (CS oligomers) has been demonstrated to induce various biological activities in plants [5] and it promoted growth in eucalyptus citriodora hookbarley and soybean[6].

4.2.6 Growth promotion effect on fresh weight of plucked leaves &thickness of betel vine

Figure 5 shows the photograph of fresh weight of 350 ppm irradiated CS treated plucked piper betel leaves after 30 days due to applying nonirradiated and irradiated CS of different concentration. The increasing values of fresh weight and thickness were found on the treated samples (table 1). The fresh weight of the leaves and thickness of betel vine of treated samples have increased maximum around 45% and 140% respectively applying 350 ppm concentration of irradiated CS solution.

The similar result was also observed for dry weight, leaves number and stem number of the plants. Higher chlorophyll content in the CS treated plants increases the ultimate growth of the plants.

Table 1: Thickness of betel vine and fresh weight of betel leaves

Samples ID	**Thickness(mm) of betel vine**			**Wet weight of matured and plucked betel leaves/ week (g)**		
	Day 1	**Day 30**	**Increased thickness (mm) than control**	**Plucked leaves from 10 plants/week**	**Total wet weight (g)**	**Average weight (g)**
Control	3.6	5.1	1.5	10	20.4	2.04
Non-irradiated CS	3.3	5.1	1.8	15	23.9	1.59
350 ppm	4.3	7.9	3.6	22	65.9	2.99
500 ppm	5	7.5	2.5	21	54.1	2.57
750 ppm	3.7	5.9	2.2	22	53.5	2.43

Figure 5. Photograph of the fresh weight of 350 ppm treated (irradiated CS) plucked piper betel leaves after 30 days of foliar spray.

As described before, the specimens were weighed after 90 days on wet basis and the results are tabulated in table1. We have observed that irradiated CS (50 kGy) have significantly higher wet weight compared to that of the plant treated by nonirradiated chtosan or the control specimen.

All data are the average of three replicate independent experiments and the standard deviation was calculated using one-way ANOVA.

4.2.7 Growth promotion effect on nutrients and heavy metals content

The effect on nutrients and heavy metals uptake by piper betel vine after 30 days due to applying nonirradiated and irradiated CS of different concentration were establish on table 6. This table also shows that in CS treated samples, heavy metals consumption is lower than control. CS is an excellent chelator of many harmful metals (e.g. Cu, Ni, Co, Cd, Pb, Hg, Zn, U, Au, etc.) and nutrients. Most of the nutrients analyzed (e.g. Fe, Zn, Na, K, Ca) showed (below in table 2) increase in contents after application of solution at 350ppm, however, Na has increased significantly at 500ppm. Nutrients of group 1 and 2 metals showed increase in contents after the CS application because they do not take part in chelate formation. As and Hg showed concentration less than 0.1 and 0.3 mg/kg respectively. At higher concentration the polymer coil up, did not present at straight chain. So they show resistant to chelation. Mechanically disassembled CS facilitate chelation. A sharp

increase in interest of using CS is to detoxify hazardous wastes. The treated soil also showed lower concentration of the minerals, which may be due to the translocation of them from the plant location.Effect of heavy metal toxicity on plants were studied by G. U. Chibuike and S. C. Obiora[7]

Table 2: Effect of nonirradiated and irradiated CS on betel vines' nutrients and heavy metals uptake after 30 days of foliar spray.

Specimen	Nutrient contents(mg/Kg)					Heavy metals content (mg/Kg)		
	Fe	Zn	Na	K	Ca	Pb	Cd	Cr
Control	3	2	14	799	135	3.9.	0.33	0.21
Non irradiated	3	2	10	700	100	3.92	0.32	0.19
350 ppm	4	4	20	1419	356	3.44	0.24	0.13
500 ppm	3	3	22	964	140	2.93	0.21	0.15
700 ppm	4	3	14	930	157	3.44	0.27	0.13

4.2.8 Effect of heavy metal polluted soil on plant growth

The heavy metals that are available for plant uptake are those that are present as soluble components in the soil solution or those that are easily solubilized by root exudates [8]. Although plants require certain heavy metals for their growth and upkeep, excessive amounts of these metals can become toxic to plants. The ability of plants to accumulate essential metals equally enables them to acquire other nonessential metals [9]. As metals cannot be broken down, when concentrations within the plant exceed optimal levels, they adversely affect the plant both directly and indirectly.

Kibra[10] recorded significant reduction in height of rice plants growing on a soil contaminated with 1 mg Hg/kg. Reduced tiller and panicle formation also occurred at this concentration of Hg in the soil. For Cd, reduction in shoot and root growth in wheat plants occurred when Cd in the soil solution was as low as 5 mg/L [11]. Most of the reduction in growth parameters of plants growing on polluted soils can be attributed to reduced photosynthetic activities, plant mineral nutrition, and reduced activity of some enzymes [12].

4.2.9 CS chelation on heavy metals

The present discovery relates to agricultural compositions for delivering metals to plants and for controlling microbial diseases in plants. Specifically, the present

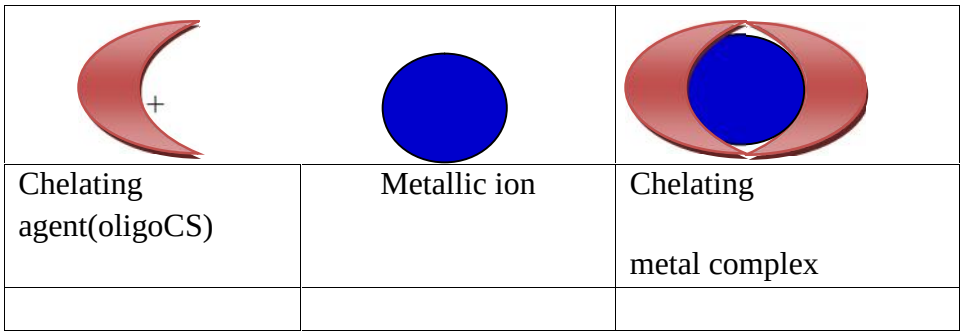
invention relates to metals chelated with CS (a particular carbohydrate-derived composition) and to methods for its use in delivering metals to agricultural crops (betel vine) and in controlling microbial damage to crops.

Historically, microbiological infestations have caused significant losses to agricultural crops and have been the cause of large scale famines and economic displacements. Fungal infections can cause pre-harvest damage to crops by killing them outright or by weakening them so as to decrease yields and render the plants susceptible to other infections. Post-harvest, fungal infections can also result in significant loss of agricultural products during storage, processing, and handling. The need for the control of microbial infections of agricultural products is well established and a number of chemical agents have been developed for this purpose, however, to date, no fully satisfactory chemical agents have been found. Oftentimes, fungal control agents are highly toxic to crops and/or animals; consequently, restrictions are placed on their handling and use. Also, many presently available fungal control agents are of restricted utility; that is to say, a particular agent may be effective only against several types of fungus. As a result, a number of separate materials must often be employed in a particular agricultural setting in order to accommodate different types of fungi or other microbial pathogens. Also, as is common with anti-microbial agents, a number of fungal species have developed resistance to commonly employed fungicides.

Clearly, there is a need for an anti-microbial control agent which can be utilized for both bacterial and fungal agents in plants which has broad activity against a variety of fungi and bacteria including those strains resistant to presently employed fungicides. Ideally, the material should be of low toxicity to crops and to animals, stable in composition, easy to employ, and preferably low in cost.

It is well known that the cell walls of fungi are comprised of chitin, which is a natural, carbohydrate-based biopolymer. Chitin is an analog of cellulose in which the OH group at the C_{-2} position has been replaced by an acetamido group. Chitin is also abundantly found in a number of natural sources, including the shells of arthropods such as shrimp. Previous research has suggested that chitin, or lower molecular weight fractions produced by its degradation, can in some instances, elicit antifungal responses in some plants [13]. CS is a semi-synthetic derivative of chitin produced by the deacetylation of the nitrogen thereof so as to produce the ammonium salt. CS itself has been shown to have some mild antifungal activity with regard to certain particular fungal species in some particular plants [14-16]. Specific hydrozylates of CS have also been described as having some antifungal activity [17] discloses the use of a composition of high molecular weight CS hydrozylate (M.W. 10,000-50,000) and acetic acid for controlling fungus in certain crops. Japanese Patent Application 62-198604 describes the use of very low molecular weight CS hydrozylates (M. W. 3,000) for the control of Alternariaalternata fungus in pears. It is further noted that this material is not effective, in pears, against other fungi such as Botrytis.

The ability of CS to form complexes with metal ions, particularly of the transition metals and post transition metal ions, is well known in the literature on George A.F. Roberts, Chitin Chemistry, Macmillan (1992)[18]. Most of the work described in this publication was done with the insoluble form of the CS metal complexes dealing with different ion interactions and the type of complex formation. Almost none of the work dealt with the soluble complex formation and no suggestion was made for the use of CS meta complexes for use in agriculture. Figure 6 shows the CS Chelate complex mechanism.



Chelating agent(oligoCS)	Metallic ion	Chelating metal complex

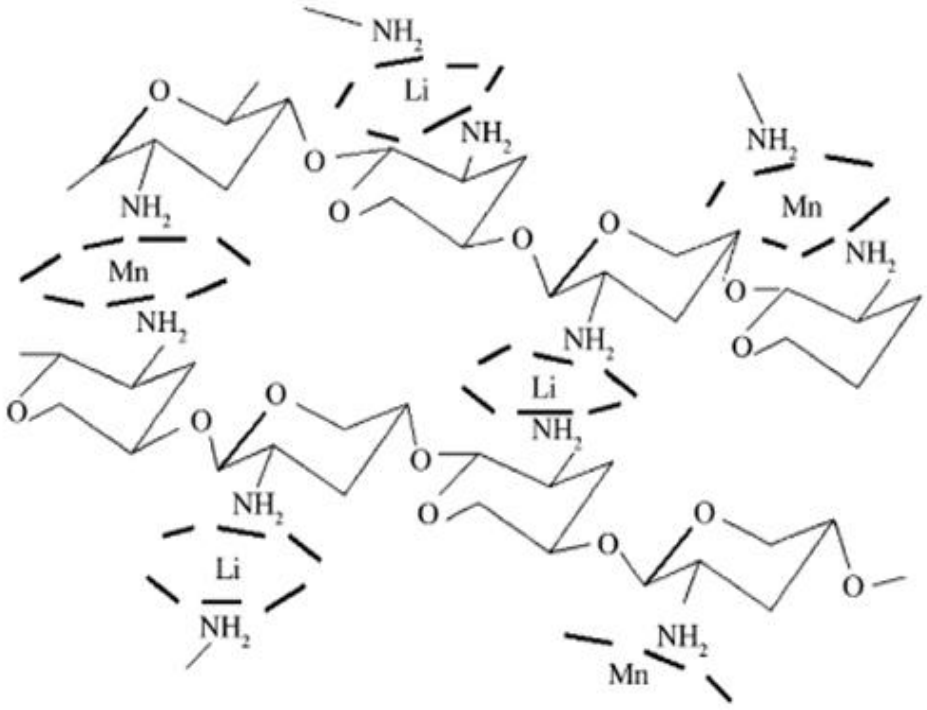


Figure 6. CS chelate complex mechanism

4.2.10 Nutrients and heavy metals content in soil of betel vine garden

Figure 7 & 8 shows the effect of nonirradiated and irradiated CS on nutrient and heavy metals content in soil of betel vine garden after 90 days of foliar spray.

It was observed in figure 7 and 8 that the transition metals have shown significant decrease in treated samples, whether Na, K and Ca showed increase in treated samples. In comparison to the data from soil samples with the plant's mineral content, it is observed that the chelate complexes did not transport to the plants. The transition metals have shown significant decrease in treated samples, whether Na, K and Ca showed increase in treated samples. In comparison to the data from soil samples with the plant's mineral content, it is observed that the chelate complexes did not transport to the plants. The treated soil also showed lower concentration of the minerals, which may be due to the translocation of them from the plant location.

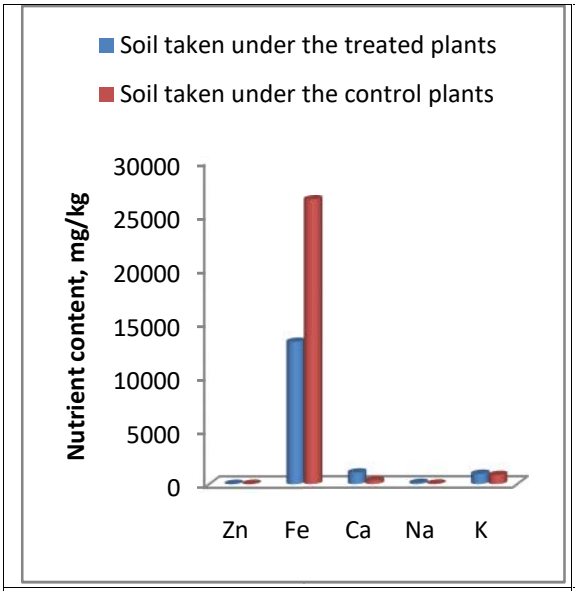


Figure 7

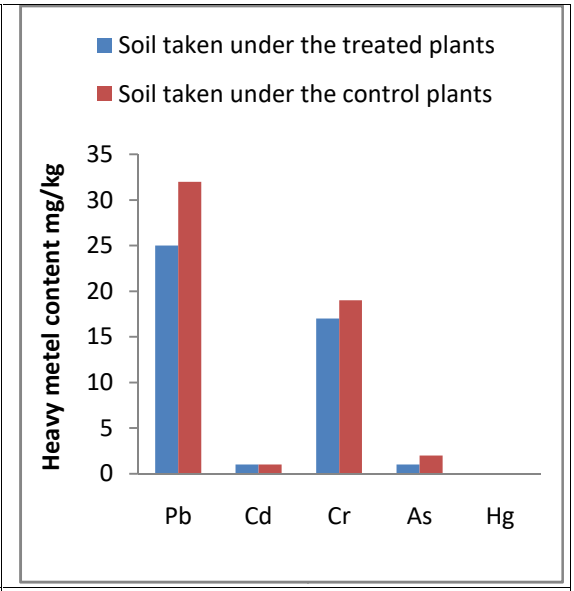


Figure 8

Figure 7 & 8. Effect of nonirradiated and irradiated CS on nutrient and heavy metals content in soil of betel vine garden after 90 days of foliar spray.

4.2.11 The sensory evaluation test (by Ranking Procedures)

Sensory analysis (or sensory evaluation) is a scientific discipline that applies principles of experimental design and statistical analysis to the use of human senses (sight, smell, taste, touch and hearing) for the purposes of evaluating consumer products.

After 30 days foliar spray of nonirradiated and irradiated CS on piper betel vine, the sensory evaluation test was conducted by ranking procedures. Samples were served with no giving any information of the differences between samples. Indirect samples, interval between tasting, orders of presentation etc. were also followed. Five samples for each concentration were presented at the same time and Rank positions 1 to 10 were given in order of increasing the degree of some attributes e.g. color, texture, appearance and taste. Rank positions (0-10) are taken for the average value where '0' was the lowest value and '10' was the highest value. Differences between totals are

tested for significance using Rank-Sum Difference tables. The leaves obtained from the sample treated at 350 ppm were passed the test scoring 10 out of 10, where the control sample in acceptance of 3 shown in table 3.

Table 3: The sensory evaluation test of irradiated CS treated piper betel leaves (by Ranking Procedures)

Person	Specimen	Color	Texture	Appearance	Taste
70	Control	4± 0.23	3 ± 0.16	3 ± 0.05	3 ± 0.08
	Nonirradiated	5 ± 0.13	5 ± 0.12	4 ± 0.28	3 ±0.24
	350 ppm	10± 0.07	10±0.32	10 ± 0.15	10± 0.12
	500 ppm	8 ± 0.09	8 ± 0.084	8 ± 0.23	9 ± 0.17
	750 ppm	6 ± 0.17	5 ± 0.31	5 ± 0.14	5 ± 0.087

Nutrient composition, physiochemical properties and its utilization as nutra tea was studied by some researcher [19].Sensory analysis of the four samples of Nutra-tea was carried out by a trained panel of 10 members. "Quantitative Descriptive Analysis" (QDA) method was employed for this purpose, using a scale of 0-15 cm. This scale was anchored at 1.25 cm on either end as 'Low' and 'High' representing 'Recognition Threshold' and 'Saturation threshold' respectively. Panelist were asked to mark the perceived intensity of each attribute listed on the score card by drawing a vertical line on the scale and writing the code number. The scores for each attribute for a given sample were tabulated, representing the judgment of individual panelists. Finally, mean value was taken for each attribute of a sample, representing the panel's verdict about the sensory quality of the product [19].

Mechanical and Sensory Evaluation of Noodles Incorporated with Betel Leaf Extract were studied by Leila Nouri et. al., 2005[20]. The sensory analysis of the noodles was accomplished in "Sensory Booths "under white fluorescent light. The booth area was kept at 20± 1°C and 50% ± 5% RH. The parameters for the noodles were made by "Free choice profiling "and were appropriately listed on the developed score card [19].

The sensory evaluation for the four noodles specimens was carried out using a method described in literature [19], with slight modifications. Briefly, 38 volunteers comprised of post graduate students and lab assistants from the Food Technology Division and other divisions of University in Malaysia were selected and trained toevaluate the parameters. A 7 point hedonic test was used for this purpose to obtain a comprehensive profile of the quality attributes of the noodles being assessed.The panelists were instructed to mark the perceived intensity of each listed characteristic by a number from 1 to 7. The scores for each characteristic for a given specimen were tabulated, representing the verdict of the individual panelists. Finally, the mean value was taken for each characteristic of a specimen, representing the judgment of the panel on the sensory quality of the noodles.

4.2.12 Growth promotion effect on reduction of diseases

Figure 9 shows thephotograph of diseased betel leaves on untreated betel vine and healthy piper betel vine after 30 days due to applying nonirradiated and irradiated CS of different concentration.

These experiments were held in Cox'sbazar district of Bangladesh. Cultivators noticed that betel vine were affected by some disease in their local language i.e. gorapocha, lotapoka, kaloversas, patakura, agapocha, shit laga, patachera, patacidro etc. After consult with the local agriculture office, Coxsbazar it was informed that there was no paan research institute. But they noticed that this perennial crop is found to be infected by various diseases of which Powdery mildew, Foot Rot & Leaf Rot caused by pathogens, Phytophthora parasitical and Colletotrichumcapsici are the major constraints for cultivation of the crop across the country. Leaf rot can damage the crop within a week when it attacks the vine. Leaf rot and foot rot have been reported to be caused by Phytophthprapalmivora and leaf rot may cause 30-100% leaf yield loss. The relative humidity enhances the incidence of the leaf rot disease. In rainy season the belel vine were also attacked more than other season. Betel farmers face reflective loss due to attack by leaf rot.

After 30 days, due to applying of nonirradiated and irradiated CS of different concentration on betel vine we got a 100% healthy and disease free piper betel vine by the treatment of 350 ppm, 50 kGy irradiated CS solution shown in figure 9.

By Kume (1997) [21], it was concluded that irradiated CS having molecular weight of 105 to 3×10^5 exhibited highly antimicrobial activities. It stimulates the plant growth and improves disease and insect resistance of plants [22]. Both chitin and CS are known to induce defence responses in plants, which include lignifications [23], ion flux variations, cytoplasmic acidification, membrane depolarization, protein phosphorylation[24], and activation of chitinase and glucanase enzymes[25]; it also induces phytoalexin biosynthesis[26], generation of reactive oxygen species[27], biosynthesis of jasmonic acid [28], and the expression of unique early responsive and defence-related genes[29].

Besides, CS and its derivatives have been widely used in medicine, biotechnological applications and in wastewater treatment [30]. CS has also been used as a coating material for seed, leaf, fruits and vegetables [31] and as plant protection tool against the microorganisms/pathogens.

CS has been demonstrated to be an effective plant protector against infectious diseases and environmental stress. Figure 9 the effect on diseases of piper betel vine after 30 days due to applying nonirradiated and irradiated CS of different concentration.

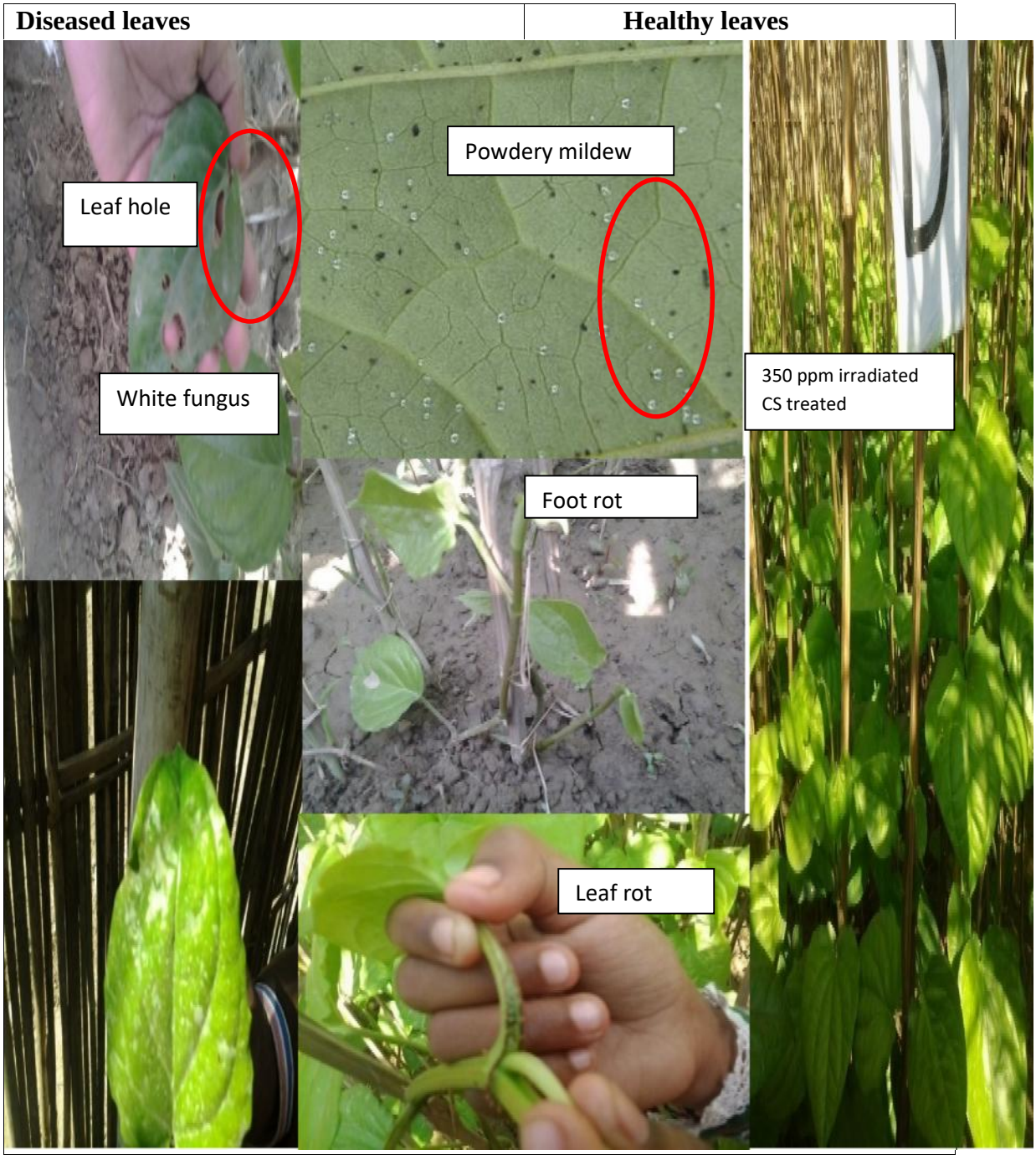


Figure 9. The effect on diseases of piper betel vine after 30 days due to applying nonirradiated and irradiated CS of different concentration

4.2.13 Scanning electron microscopy of pure and irradiated CS samples

The SEM images of the control betel leaves (Figure 10a) and irradiated CS (Figure 10b, c and d) are shown in figure 10. There have no significant effect on SEM image of betel leaves after the 30 days foliar spraying of CS on betel vines.

Figure 10 a (Control betel leaf)	Figure 10b (350 ppm CS treated betel leaf)

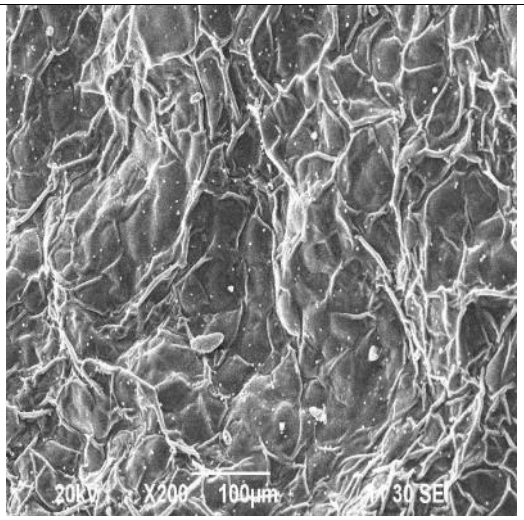
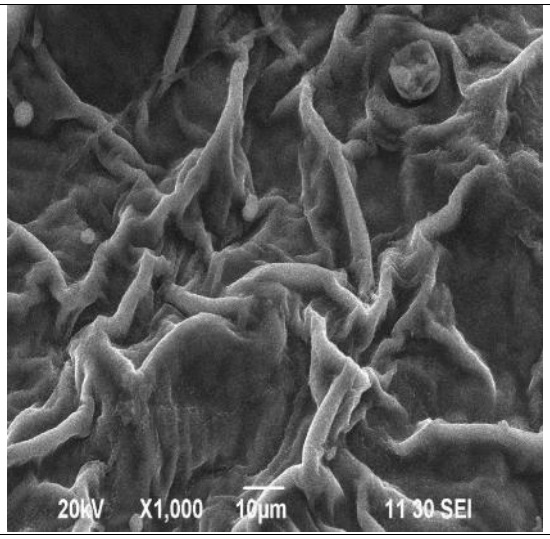

	

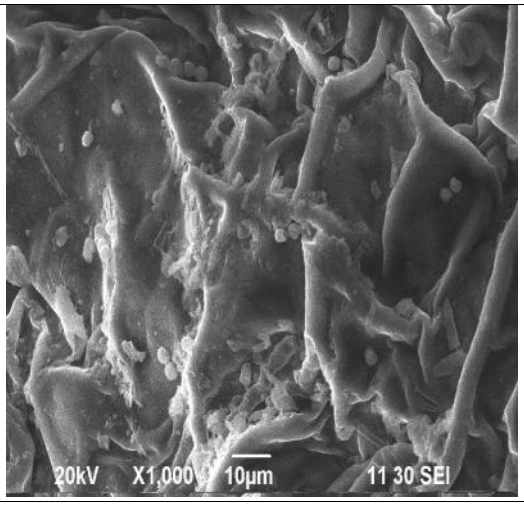
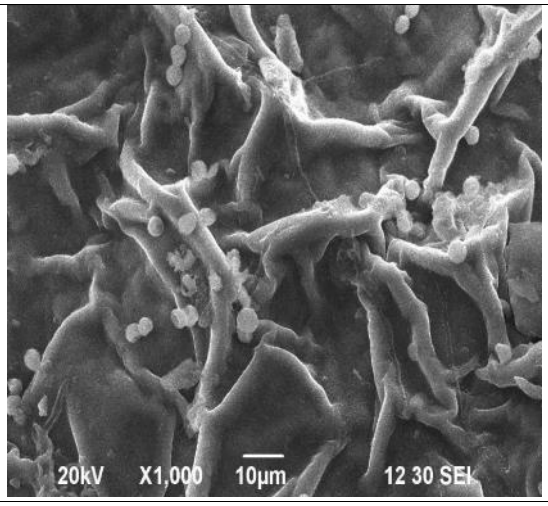
Figure 10c (500 ppm CS treated betel leaf)	Figure 10d (750 ppm CS treated betel leaf)
	

Figure 10. SEM photograph of CS trated betel leaves

4.2.14 TG analysis

Table 4 represents the weight retain percentage of control and different concentration of 50 kGy irradiated CS treated belel leaves. As seen from the results, the weight retain took place in two stages. The first one starts below 120 °C, it assigned to loss of water molecules that interact with OH and –COO– polar groups in CS chain by hydrogen bonding, since a considerable amount of water is released at temperatures below 250 °C. The second stage starts at 350 °C and reaches a maximum at 600 °C corresponds to the decomposition (thermal and oxidative) of CS. It was observed that there is a trend of reduction in the thermal stability of irradiated CS. However the weight retain percentage of irradiated CS is a little different from control and the changes in weight loss of irradiated CS are not significant at different doses applied.

Table 4: The weights retain percentage of control and different concentration of 50 kGy irradiated CS treated belel leaves.

Sample ID	Weight retain (%) at various temperatures (°C)							
	0-25°C	100-110°C	200-250°C	300-350°C	350-420°C	400-450°C	500-550°C	550-600°C
Control	100%	94%	89%	66%	62%	52%	24%	22%
350 ppm	100%	94%	87%	63%	53%	37%	22%	20%
500 ppm	100%	94%	87%	63%	53%	37%	22%	20%
750 ppm	100%	94%	87%	63%	53%	37%	22%	20%

4.2.15 Anti-fungal test

The betel vine plant farmers' faceing heavy loss due to leaf rot of pan caused by var. Fungus was counted in various petri plates after incubation. Total numbers of fungi were calculated by colony counter in PDA media.

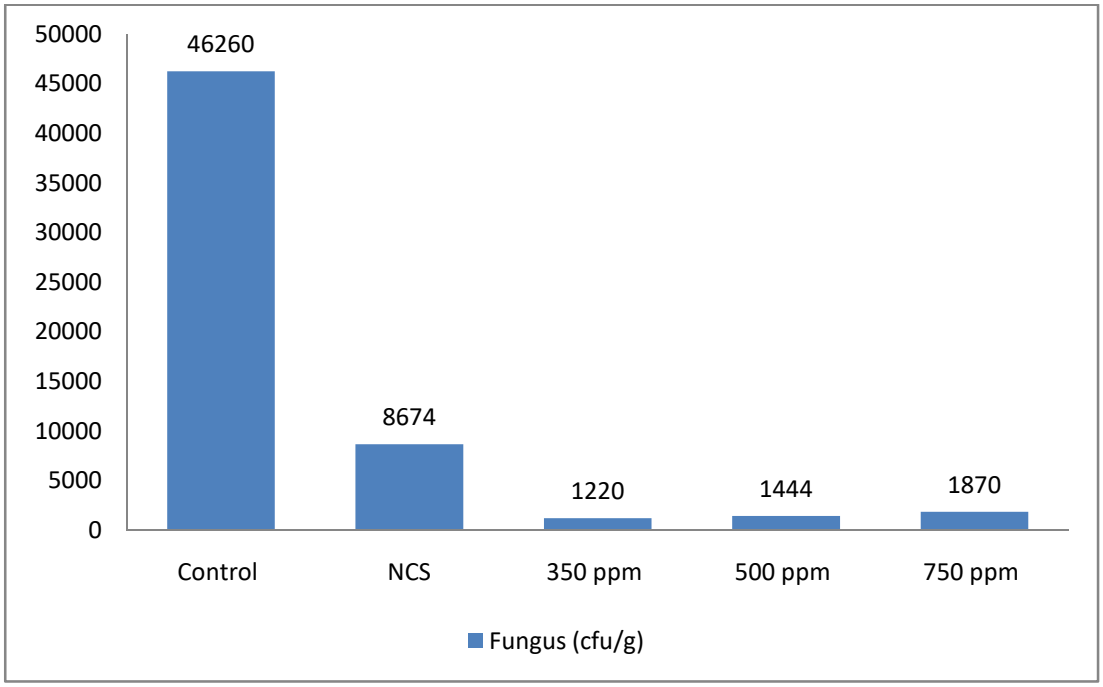


Figure 11. Anti-fungal activity of irradiated chitosan of betel leaves

From figure 11, fungal counts for 50 kGy chitosan solution at different concentrations are ranked as follows:

Control > NCS>750ppm >500ppm >350ppm

From the figure it is clear that, at 50 kGy radiation doses 350ppm chiosan solution concentration shows maximum antifungal activity in contrast with the other treatment and control plants. For control the total fugal count was 462.6×10^2, which is reduced

to 86.74×10^2 when plants are treated with nonirradiated chitosan (NCS). Effect of 350 ppm. on betel vine, total fungal count was decreased the best (12.2×10^2).

4.2.16 Shelf life of CS treated betel leaves

After 30 days foliar spraying of irradiated and NCS solution on piper betel vine the leaves were collected in an airtight bag and preserved them in the freeze of Bangladesh Atomic Energy Commission. After one month, it was observed that containing water in control samples were release greater than treated samples (figure 12). Except this, discolored and damaged percentage was also showed greater in control leaves.

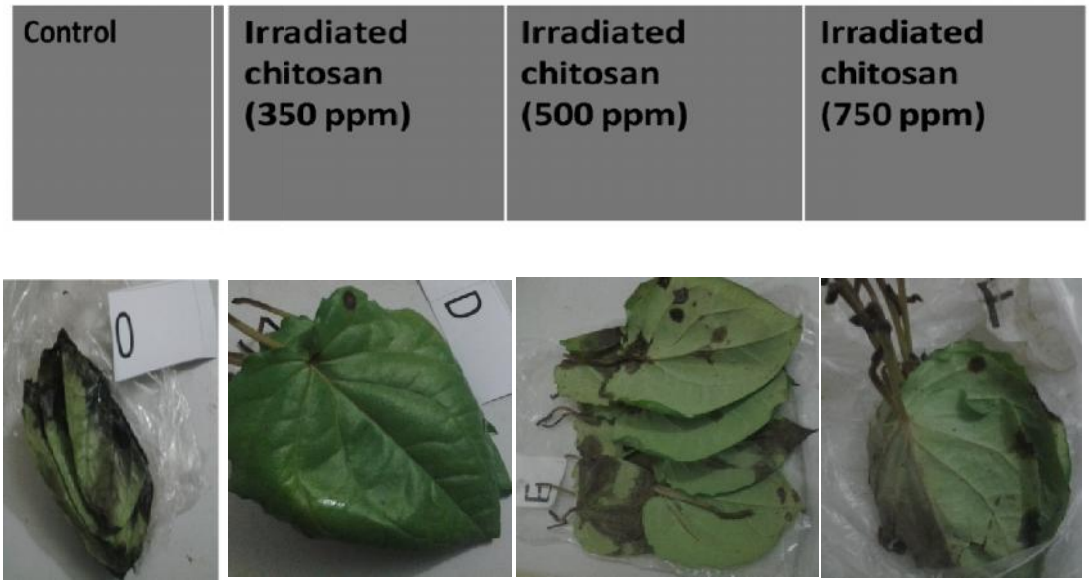


Figure 12. The effect on shelf life of piper betel vine after 30 days due to applying nonirradiated and irradiated CS of different concentration.

The effect on shelf life of piper betel vine after various treatment of 50 kGy CS solution 500 ppm treated leaves prolong their shelf life of 100% upto 30 days and which is ranked in table 5 as follows:

Shelflife: 350 ppm>500ppm>750ppm>Control

Table 5: Shelf life of CS treated betel leaves

Sample ID	**Damaged %**
Control	100%
350 ppm	0%
500 ppm	30%
750 ppm	30%

The irradiated CS showed an increase in shelf life of betel leaves after 30 day trial. CS is well known coating material used in several fruits for prolonging their shelf life [32]. The use of oilgoCS as food preservative achieved a 10 day shelf life extension [33, 34]. CS is a microbicidal and it protect fruits and vegetables from attack of Penicillium spp. Aspergillus spp., Rhizopusstolonifer and Botrytis cinerea [34].CS is a natural antibacterial polymer that keeps fruit fresh longer.

CS induces chitinase, a defense enzyme, which catalyze the hydrolysis of chitin, a common component of fungal cell walls, thus preventing the growth of fungi on the fruits. Thus CS can extend the shelf life and decrease the spoilage of fruits. Oligo-CS has been recognized as potent phyto-alexin inducer to resist infection of disease for plants [35] have ascribed the function of high molecular-weight CS as an antimicrobial material or flocculent to either amino groups in the molecule or hydrogen bonding between CS and extra cellular polymers in addition to an electrostatic interaction with the cell surface.

4.1.17 Observations

- After 50 kGy radiation dose, the molecular weight of chitosan was achieved 90000 Da and DD% was achieved 78%.
- Betel vine showed increases of plant height, number of leaves, number of stems from 50- 200% in different cases. In the last day of the experiment, the growth based on height were measured and 50 kGy irradiated CS sprayed specimens yield a growth about 67 % to that of the control.
- It was observed that 500ppm irradiated CS solution concentration showed the best efficiency on the productivity of betel vine.
- There is no significant change observed due to concentration on shoot growth.
- At 350 ppm leaf's area has increased highest (78%).
- The fresh weight of the leaves and thickness of betel vine of treated samples have increased maximum around 45% and 140% respectively applying 350 ppm concentration of irradiated CS solution.
- CS treated samples; heavy metals consumption is lower than control. CS is an excellent chelator of many harmful metals (e.g. Cu, Ni, Co, Cd, Pb, Hg, Zn, U, Au, etc.) and nutrients. Most of the nutrients analyzed (e.g. Fe, Zn, Na, K, Ca) increase in contents after application of solution at 350 ppm, however, Na has increased significantly at 500ppm.
- Transition metals (Pb, Cd, Cr, As, Hg) have shown the significant decrease, whether Na, K and Ca showed an increase in the irradiated CS treated area of soil of the betel vine garden.
- Completely healthy and disease free piper betel vine garden was observed after 30 days, due to applying of irradiated CS on betel vine and significant reduction of diseases was observed on betel vine after the treatment of 350 ppm, 50 kGy irradiated CS solution.

- The weight retain (%) of irradiated CS treated betel leaves and control was found on more or less same; there was no significant change was observed for TG analysis. The changes in SEM image of irradiated CS treated betel leaves and control leaves are also alike.
- Effect of 350 ppm on betel vine, total fungal count was decreased the best at (12.2×10^2).
- The effect on shelf life of piper betel vine after 30 days various treatment of 50 kGy, 500 ppm CS solution, treated leaves prolong their shelf life completely than all other treatment as well as control. Sensory evaluation test ranked 10 out of 10 for chitosan applied sample whereas the control ranked 3 only.
- This study suggests that gamma irradiated Chitosan can be used as plant growth promoter. The growth promotion activity of the oligomer chitosan varies on crop varieties which mean dose optimization is required on a particular plant. The chelating capacity of chitosan reduces the heavy metal

uptake of the plants from the soil as well as increase the nutrient uptake for the plant. CS is a natural microbicidal and thus reduces the attack of diseases on plants and also preserves fruit and vegetables longer and safer than commonly used synthetic preservatives.

4.2.18 References

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Chapter 5:Results and Discussion

(Section 5: Application of irradiated SACS on betel vine)

5. Results & Discussion

Section 5: Application of the binary mixtures of irradiated SA and CS on betel vines

5.1 Introduction

Recently, there is a tremendous potential for using polymers in agriculture. In the last decades functionalized polymers revolutionized the agricultural, horticultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients etc. [1]. There is a worldwide trend to explore new natural products that act as growth promoters for plants and that control postharvest pathogenic diseases, giving priority to that enhance the plant productivity, reduce disease incidence and avoid negative and side effects on human health as a result of the excessive application of synthetic agrochemicals. Among of them CS, SA or carrageenan, a high molecular natural polymer, nontoxic, bioactive agent has become a useful appreciated compounds due to its bio-fertilizer, promotion of germination and shoot elongation,[2] as a growth stimulator on growth and yield of rice, wheat, maize, black pepper, bean, cabbage, peanut, soybean, tomato, cotton, strawberry,[3-5] in orchid tissue culture,[6] fungicidal effects or elicitation of defense mechanisms in plant tissues,[7-10] stimulation of growth of bifidiobacteria to resist infection of diseases for plants particularly oligoCS in agriculture as biotic elicitor to enhance defense responses against diseases[11] and suppression of heavy metals stress[12]. Different processing technologies have been applied to transfer natural polymers into the marketable products.

Radiation processing offers a clean and additive free method for preparation of value-added novel materials based on renewable natural polymers which can be used in various applications including health care, food, polymer processing industry and environment. To fulfill the demands of specific applications, the natural polymers need to possess different characteristics, for example, while for agricultural applications, radiation processing should lead to the formation of lower molecular products. For the environmental applications demand the formation of crosslinked network structures. Nowadays, radiation modification and degradation of natural polymers to obtain low molecular weight polysaccharides or oligosaccharides were used for development of new applications. Irradiation of SA or CS led to the reduction of molecular weight by scission of glycosidic linkage [13-15]. SAs are the major components of brown seaweed cell walls. Oligo-SA has been obtained by digestion of SA with an SA, lyase, by treatment with γ-radiation, or by acid hydrolysis. In particular, it has been shown that SA, depolymerized using γ-radiation, at concentrations of 0.5–1 mg/ml, enhance growth of rice and peanut plants cultivated hydroponically [16].In addition, a mixture of oligo-SA sobtained by degradation of SA with a bacterial SA lyase, at a concentration of 0.5–3 mg/ml, stimulated growth of

roots in lettuce, [17] stimulated elongation of carrot and rice.[18]. SA prepared by degradation of SA with γ-radiation, at a concentration ranging from 0.02 to 0.1 mg/ml, increased shoot and root length, shoot dry weight, content of total chlorofills catenoids, nitrate reductase activity involved in nitrogen assimilation and alkaloid contents, mainly morphin and codeine, in opium poppy plants [19].

CS is one of the most important marine polysaccharide has many peculiar biological activity ssuch as immunity, nor cholesterol and antibacterial [20, 21]. Degradation of CS is usually used, turning CS in to one with low molecular weight which exhibits good water solubility. The water-soluble CS with low molecular weight has some special biological, chemical and physical properties which are different from that of the ordinary CS such as antibacterial activity, [22] antifungal activity [23] and antitumor activity [24]. Due to many unique properties such as biocompatibility, biodegradability, nontoxicity and non antigenicity; CS has been widely applied in medicine, biotechnology, water treatment, agricultural and food science [25]. Oligo-CS has received much attention as an alternative value-add end product because of their bioactivity and other novel biological properties [26]. The main objective of the research was to elaborate the method of γ-irradiation of sodium SA and CS to get different oligomers and studying the effect of their mixtures on the growth promotion and productivity of betel vine. From the results presented in the chapter 3.1, 3.2, 4.1, and 4.2, it is noticed that after foliar spraying of irradiated SA on selective crops and

Table 1: The effect of foliar spraying of irradiated SA and CS on selective crops and betel vine.

Parameter observed	**Selective crops**	**SA(Best results)**	**Selective crops**	**CS (Best results)**
Plant height	Mung dal Betel vine	300% 72%	Mung dal Betel vine	340% 67%
Shoot growth	Chilli Betel vine	200% 250%	Jute Betel vine	225% Not significant
Number of leaves	Red chilli Betel vine	300% 96%	Red chilli Betel vine	350% 67%
Leaves area	Pumpkin Betel vine	110% 100%	Jute Betel vine	207% 78%
Number of fruits	Green Chilli	300%	Tomato	371%%
Size of fruits	Egg plant	201%	Cauliflower	302%
Root length	Egg plant Betel vine	300% 30%	Tomato Betel vine	292% 45%

and betel vines, significant increase in different parameters were observed in different plants and these higher in comparison to those of irradiated CS treated crops. The plant height, shoot growth, number of leaves, leaves area, root length, number, and size of fruits were observed after application of the SA and CS on the crops and the betel vine (table 1). CS treated crops and vines showed comparatively less increase in the plant parameter studied. However, CS treated betel leaves showed a longer shelf life, complete protection against disease and ranked 1`0 out of 10 in sensory test. In this perspective, it has been decided to mix both the polymers for spraying on betel vines to achieve better plant of properties with better shelf life and better in taste.

CS treated crops and vines showed comparatively less increase in the plant parameter studied. However, CS treated betel leaves showed a longer shelf life, complete protection against disease and ranked 1`0 out of 10 in sensory test. In this perspective, it has been decided to mix both the polymers for spraying on betel vines to achieve better plant of properties with better shelf life and better in taste.

5.2 Growth promotion test for the mixture of SACS

SA and CS were prepared by γ-irradiation at and respectively. The effects of foliar spraying of 12 kGy irradiated SA and 50 kGy irradiated CS on betel vine was described in the previous chapter 3.2 and 4.2. Spraying of various concentrations of irradiated SA and CS on betel vine plants was proved a positive effect on plant growth and the productivity at the same time. This chapter 5 deals a comparative study of the binary mixture of SA and CS. For that, three different ratios (1/1, 2/1, 9/1) of SA/CS respectively were prepared. The effects of spraying of the binary mixture of SA and CS on growth promotion behavior were evaluated using betel vine. After plantation of three months the binary mixture of SACS were started for foliar spraying on betel vine plants for 30 days at 7 days of intervals. Each group divided into different separate lines. Plants were sprayed by 1/1, 2/1 and 9/1 ratios of SACS using 350 ppm, 500 ppm and 750 ppm solution concentration of SA and CS. The test field was divided into four groups for the concentration of 350 ppm, 500 ppm, 750 ppm and control. 10 plants were taken for each concentration of solution. Thus 100 plants were taken for this experiment (90 plants for three concentration and 10 for control). Solutions needed for one type of ratio is 500 ml. Samples were prepared by this type:

- Each of the ratios was prepared for the concentration of 350 ppm, 500 ppm and 750 ppm.
- For ratio 1/1 of SA/CS, the calculation of 350 ppm, 500 ppm and 750 ppm will be termed as A, B and C respectively.
- For ratio 2/1 of SA/CS, the calculation of 350 ppm, 500 ppm and 750 ppm will be termed as D, E and F respectively.
- For ratio 9/1 of SA/CS, the calculation of 350 ppm, 500 ppm and 750 ppm will be termed as G, H and I respectively.

5.3 Growth promotion effect on plants height

Figure 1 shows that the changes of plant height of betel vine plants after 30 days due to applying SACS with different ratio (1/1, 2/1, 9/1). After three months of plantation, the specimen plants of piper betel vine were treated with irradiated SACS. It clearly shows that the treated samples had a much more height compared to control samples on different days and the test specimens treated with 2/1 ratio of 350 ppm solution concentration of SACS had highest enhancement of betel vines' height after 30 days.

Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration plant height increased 43%, 48%, and 45% respectively than control (36%). Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration plant height increased 57%, 56%, and 55% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration, plant height increased 55% and 52% and 51% respectively than control. It was revealed that 2/1 ratio of SACS at 350 ppm was found to increase the best plant height of betel vine plants at a rate of about 57%.

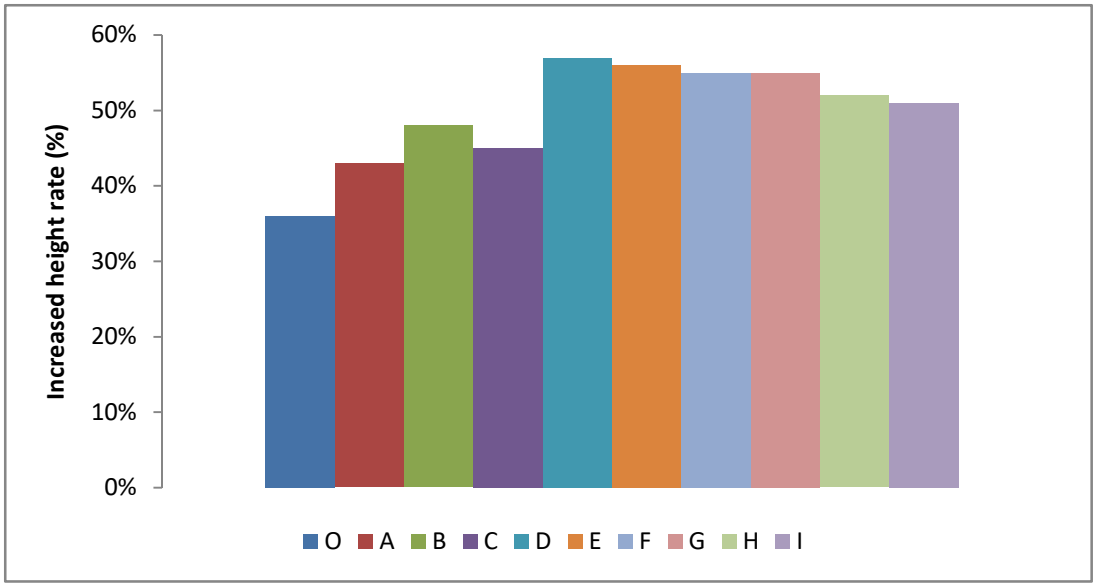


Figure 1. Effect of irradiated SACS on betel vines' height after 90 days of foliar spray.

Similar results were found where researcher obtained a promising effect on the growth, after spraying with binary mixture especially the ratio of (2/1) of oligo-SA/ oligo-CS on zea maize plants[15]. The results are agreed well with previous reports [15-16, 27-30]. The mixture of oligoSA/ oligo-CS with ratio of (2/1) prepared at 45 kGy gives plant length 313cm, whereas control one give up 266cm[15].

The effect of foliar spraying of 350 ppm, 500 ppm, 750 ppm of SA, CS was explained in the previous chapter. It was obvious that the treatment of betel vine plants with the SA and CS oligosaccharides obtained by γ-irradiation enhances betel vine's plant

growth promotion and performance. From the previous chapters, it was also concluded that the higher plant height was obtained for those plants treated by the irradiated SA than irradiated CS. The lower molecular weight of SA obtained by γ-irradiation, the higher the growth promotion effect.

5.4 Growth promotion effect on plants number of leaves

Figure 2 represents the effects of irradiated SACS on betel vines' leaves count. It exhibits that, the effectiveness in three types of experiments (1/1, 2/1, and 9/1 ratio of SACS) has increased the leaves count compared to untreated samples. In case of 2/1 ratio of SACS mixture at 500 ppm solution concentration, matured betel leaves were also higher (123.52%) than other ratios. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm,500 ppm and 750 ppm concentration increased number of leaves is found 41%, 100%, and 83% respectively than control (24%). Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration increased number of leaves found 147%, 135%, and 124% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration, increase the number of leaves found 124% and118% and 100% respectively than control. The highest leaves (147%) were obtained due to the foliar spraying of 2/1 ratio of the SACS at 350 ppm concentration.

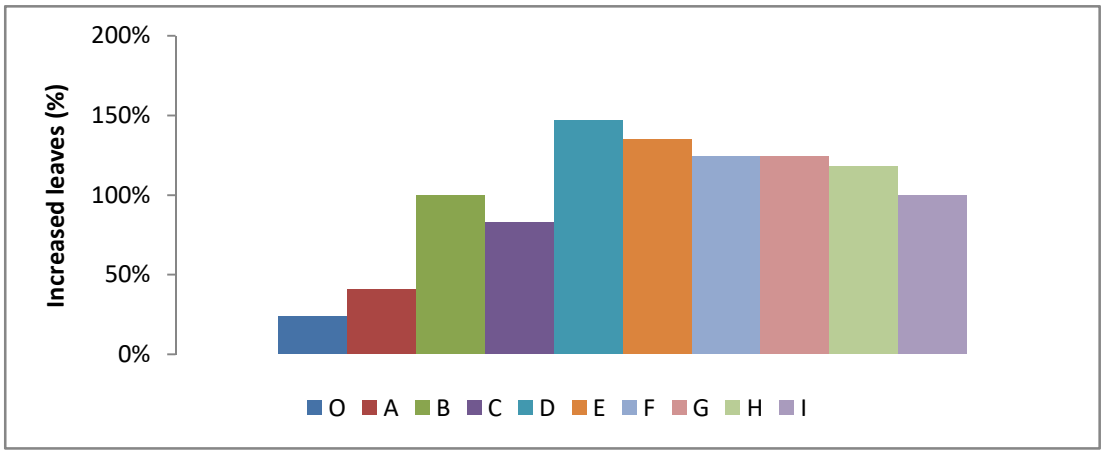


Figure 2. Effect of irradiated SACS on betel vines' number of leaves increased rate after 30 days of foliar spray.

Similar results found in Zea maize plants [15]. Compared to control plants, they also found the increase grain yield (%) of crop Zea maize by treatment of oligoSA prepared at 25, 35 and 45kGy was 8.6, 31.7and 47.3 %, respectively. While, the increase in grain yield (%) by the treatment of oligo-CS was 5.5, 26.2 and 40.6 %, respectively [15]. They also found with increasing the ratio of oligo-SA in the binary mixture increases the yield. The mixture of oligo-SA/ oligo-CS prepared at 45kGy with the ratios of (1/1), (1/2) and (2/1) enhance the increase in yield crop as followed 39.8, 48.1, 53.9%, respectively. The results have similarities with previous reports [15-16, 28-30]. After irradiation, the resultant oligo-CS and oligo-SA solutions were directly

used as a biotic elicitor for plants. The effect of mixing the oligo-SA has a promising effect on increasing the grain yield crop.

5.5 Growth promotion effect on plants' shoot/stem production

Figure 3 shows the increased shoot growth value obtained due to the 30 days foliar spraying of SACS on betel vine. It clearly shows that all concentration and all ratios of SACS were proved the enhanced value of leaves count on betel vine. This figure also proved that leaves got due to the foliar spraying of SACS mixture due to the foliar spraying of 2/1 ratio of 350 ppm solution concentration which had been reduced with 1/1 and 9/1 ratios. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm and 750 ppm concentration increase the shoot growth is more or less same to the control (100%). At 500 concentration shoot growth increased to 150% as compared to the control. Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration increased shoot growth found 300%, 250%, and 200% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration shoot growth increased as regards200% and150%, respectively than control. Effect of foliar spraying of 2/1 ratio of 350ppm SACS solution on betel vine, were found to increase the best productivity on the stem at a rate of about 300% give up due to 30 days of foliar spraying.

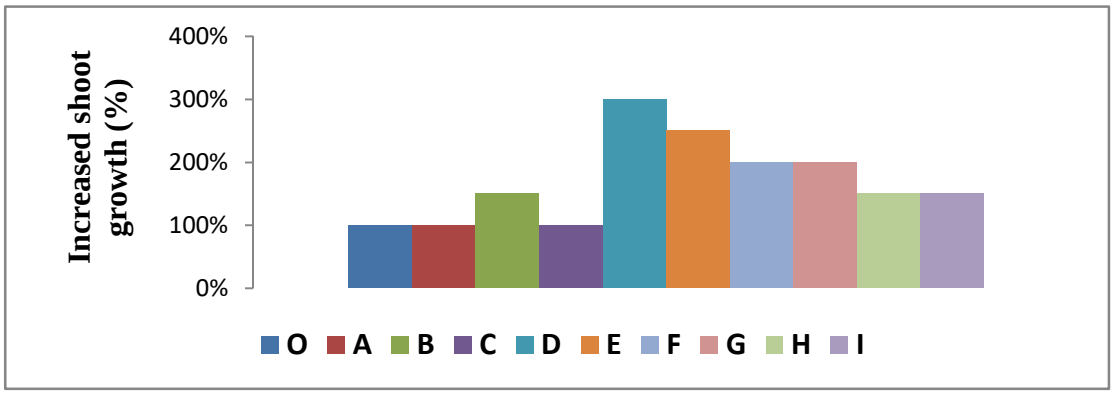


Figure 3. Effect of irradiated SACS on betel vines' number of stems increased rate after 90 days of foliar spray.

Researchers concluded that SA oligomers generated by de-polymerization of SA have been reported to stimulate the plant growth, seed germination and shoot elongation in plants [16]. They act as signal molecules that regulate plant growth and development as well as the defense reactions in plants by regulating gene expression.

The results suggest that SA derived oligosaccharide probably applied as leaf-sprays improved the growth attributes, enhanced the acceleration of them metabolic activities, photosynthetic capability and enzyme activities.

5.6 Growth promotion effect on vines' thickness

Figure 4 showed the effect on vines' thickness due to the 30 days foliar spraying of SACS on betel vine. It clearly shows that the treated samples had a much more thickness value compared to control samples on different days and the test specimens treated with 2/1 ratio of 350 ppm solution concentration of SACS had the highest enhancement of betel vines' thickness after 30 days. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration increase the vine's thickness as regards 67%, 73%, and 70% respectively whereas control plants increased this value of 41%.Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration increase the vine's thickness as regards 140%, 133%, and 100% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration, increase vine's thickness as regards 94%, 80%, and 74% respectively than control. This result obtained by treatment with different ratio of irradiated SACS showed increased thickness value. 2/1 ratio of SACS solution at 350 ppm was proved to increase the highest thickness value at a rate of about 140% whereas control increases only 41%.

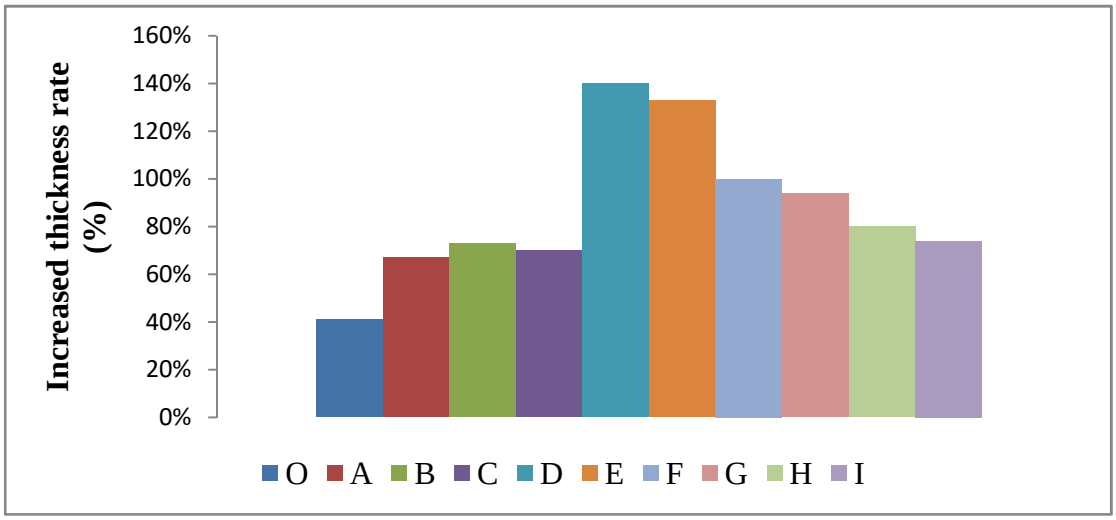


Figure 4. Effect of irradiated SACS on betel vines' thickness increased rate after 30 days of foliar spray

In a previous report [15], the grain yield/acre during the treatment of plants by γ-irradiation SA or CS (solid form as a paste) in presence of 10% H_2O_2 at 120 kGy was 26.7 and 25.8 ardab, respectively. While, in their experiment, 5% SA or CS solutions containing 1% H_2O_2 was degraded by γ-irradiation at 45 kGy enhanced to 35.5 and 32.8 ardab, respectively with an increase about 33 and 28 % in the yield, respectively. It was reported that the oligo-SA prepared by irradiation promoted the growth and development of crop plants (rice, peanuts, barley, and soybeans) [16].

CS has a positive impact on plants growth. It was reported that irradiated CS solution is effective as plant growth enhancer. CS enhances the vegetative growth in terms of

the average values of stem length, a number of growing leaves, including leaf width and length. The use of irradiated SA improved the quality of maize plant. The grain size, grain weight, total protein and total oil percentage increased if compared with the control one. SA has been reported to stimulate the plant growth and seed germination in plants [23-24].

The results suggest that foliar spraying of irradiated SA on leaf improved the growth attributes, accelerate photosynthetic capability and enzyme activities [24]. SA and CS act as signal molecules that regulate plant growth and development as well as the defense reactions in plants by regulating gene expression.

5.7 Growth promotion effect on betel leaves area

The results obtained by treatment with various ratios of irradiated CS and sodium SA mixture showed increased leaves area of betel vines than that of the control (figure 5). It indicates that treated specimen had a greater enhance rate.

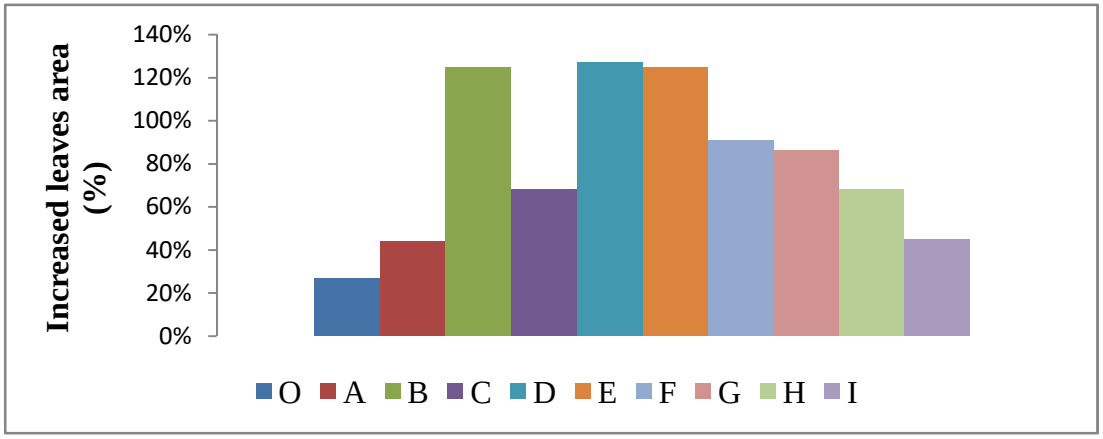


Figure 5. Effect of irradiated SACS on leaves area after 30 days of foliar spray

Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration increase the area of leaves 44%, 125% and 68% respectively compared to those of the control. Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration increase the area of leaves as regards 127%, 125% and 91% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm, 500 ppm, and 750 ppm concentration increase area of leaves as regards 86%, 68%, and 45% respectively than control. The mixture of 2/1 ratio of SACS solution at 350 ppm was found to increase the best leaves area of betel plants at a rate of about 127% whereas control increases 27%.

It was reported that the oligo-SA prepared by irradiation promoted the growth and development of crop plants (rice, peanuts, barley, and soybeans) [16]. It was reported that irradiated CS solution has a positive impact on stem length, number of growing leaves, including leaf width and length. The use of irradiated SA improved the quality

of maize plant. The grain size, grain weight, total protein and total oil (%) increased if compared with the control one. The SA oligomers generated by de-polymerization of SA have been reported to stimulate the plant growth, seed germination and shoot elongation in plants [25-26].

5.8 Growth promotion effect on fresh weight of betel vines' leaves

From table 2 it has shown that, all concentration and ratios of SACS solution gainedhigher weight than control. After three months of plantation, the specimen plants of piper betel vine were treated with irradiated SACS. It clearly shows that the treated samples had a much more weight compared to control samples on different days and the test specimens treated with 2/1 ratio of 350 ppm solution concentration of SACS had highest wet weight of betel leavess' height after 30 days.

Table 2: Effect of irradiated SACS mixture on betel leaves wet weight after 30 days of foliar spray.

Treatment	ID	Matured and pluck able leaves from 10 plants	Total wt.(g)	Average wt. (g)	Increased weight (%)
Control	O	10	20.4	2.04	-
1/1 ratio	A	14	25.5	1.82	12%<O
	B	14	26.9	1.92	6%<O
	C	12	20.2	1.68	21%<O
2/1 ratio	D	14	20.3	1.45	40%<O
	E	14	24.7	1.63	25%<O
	F	12	20.4	1.70	20%<O
9/1 ratio	G	16	33.12	2.07	1.47%>O
	H	16	34.4	2.15	5.39%>O
	I	15	30.75	2.05	0.49%>O

Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration decreases the wet weight value of leaves as regards 12%, 6% and 21% respectively as compared to control. Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm, 500 ppm and 750 ppm concentration also decreases the wet weight value of leaves as regards 40%, 25% and 20% respectively than control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration, increase the wet weight value of leaves as regards 1.47%, 5.39%, and 0.49% respectively than control. In case of 2/1 ratio, the highest weight gain (5.39%) of fresh betel leaves were obtained (2.15g/leaf) from 350 ppm concentration treated matured leaves. There was no significant effect observed on fresh weight of betel leaves due to 30 days foliar spraying of variatious ratios of SACS.

Similar results observed in the mixture of oligo-SA/ oligo-CS with ratio of (2/1) prepared at 45 kGy gives increased the dry weight of grain yield of zea maize plants compared with control. In their experiment, 5% SA or CS solutions containing 1% H_2O_2 was degraded by γ-irradiation at 45 kGy enhanced to 35.5 and 32.8 ardab respectively with an increase about 33and 28% in the yield, respectively [15].

The grain size, grain weight, total protein and total oil (%) increased compared with the control one. The SA oligomers generated by de-polymerization of SA have been reported to stimulate the plant growth, seed germination and shoot elongation in plants [16].The use of irradiated SA improved the quality of maize plant [27]. Irradiated CS solution is enhances the vegetative growth of plants.

5.9 Growth promotion effect on betel vines' root's length

Figure 6 presented the results obtained by treatment with various ratios of irradiated SACS mixture showed increased root value. It was observed that, all ratios shows increased roots value compared to the control but 1/1 ratio of SACS at 350 ppm were found to increase the best roots' length value with compared to other ratios and control. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration increase the roots value as regards 97%, 100% and 95% respectively than control (39%). Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm,500 ppm and 750 ppm concentration the root valueincreased 74%, 71% and 63% respectivelythan control. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration, the root valueincreased 54%, 45%, and 42% respectively than control. In this figure is was observed that 1/1 ratio of SACS at 350 ppm were found to increase the best roots' length value of about 100% with compared to other ratios and control (39%).

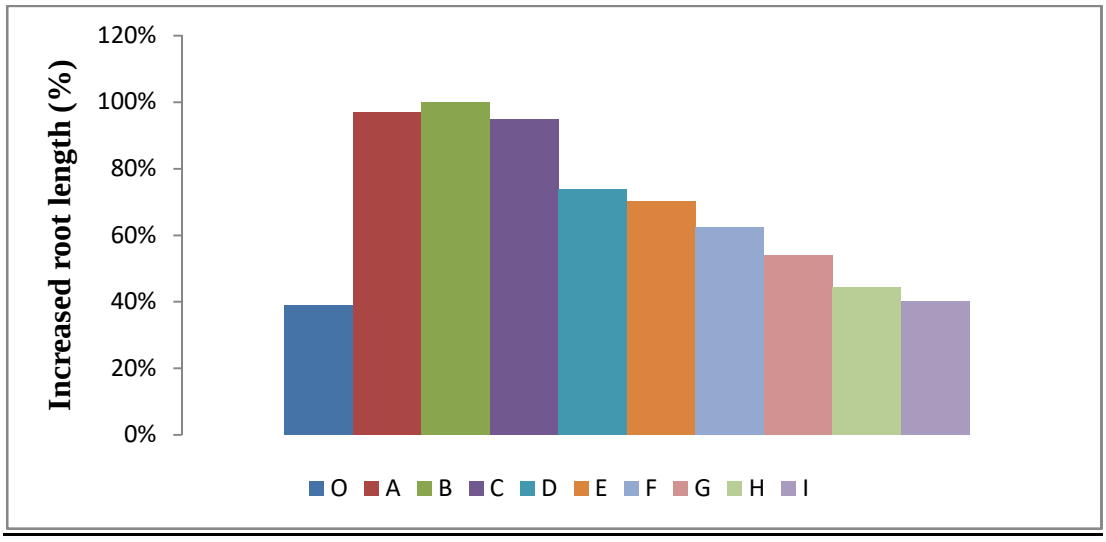


Figure 6. Effect of irradiated SACS on betel vines' root's length increased rate after 30 days of foliar spray.

CS in solid state was irradiated with electron beam from an electron beam accelerator. Elektronika 10-10 with a dose range from 50 to 300 kGy [28]. The effects of irradiation on the molecular weight of CS were investigated by viscosity and GPC measurements. Non-irradiated and irradiated CS at concentrations 0.001,0.01, 0.1 and 1 g/dm3 were used for green house test sof its activity for growth promotion of Salix viminalisL.var.giganteaplant. Uniform rooted cuttings (20 per combination) were selected for the test and cultivated in aeratedhy droponics culture containing Hoagland's nutrient solutions plus respective amounts of CS. After six weeks of plant exposure to CS, data of selected parameters of plant growth were collected. Inmostcases, except the highest concentration, both forms of CS had stimulatory effect on leaf area, length of roots and of newly developed shoots. Also fresh and dry weights of these organs were greater in CS treated plants. The highest concentration of CS was stimulatory only for a number of roots and newly developed shoots while for other parameters were inhibitory. In the stimulatory effect was greater for CS irradiated in comparison with the non-irradiated one.Researcher revealed that a mixture of oligo-SA sobtained by degradation of SAs with a bacterial SA lyase, at a concentration of 0.5–3 mg/ml, stimulated growth of roots in lettuce,[17] stimulated elongation of carrot and rice.[18]. CS with different molecular weights was investigated as a bio stimulator abiologically active substance that stimulates somegrowth processes in plants.

5.10 Nutrient uptake on betel vines

The effect on nutrients uptake by piper betel vine after 30 days due to applying irradiated SACS of different concentrations and ratios were established on table 3. Nutrient consumption in betel leaf is higher than control in all concentrations of 1/1 and 2/1 ratio of SACS treated betel leaves. Most of the nutrients analyzed (e.g. Fe, K,

Table 3: Effect of irradiated SACS on betel vines' nutrient uptake after 30 days of foliar spray.

Treatment	ID	Nutrient uptake (mg/kg)				
		Fe	Zn	Na	K	Ca
Control	O	3.83±0.38	3.82±0.01	21.95±0.86	1418.87±17.03	134.30±1.88
1/1 ratio of SACS	A	4.12±0.09	4.66±0.02	17.99±0.02	1428.15±2.33	203.22±1.55
	B	4.53±0.15	4.15±0.01	16.44±0.12	1426.08± 11.76	203.19± 3.52
	C	4.36±0.12	5.44±0.02	14.12±0.01	1426.04±11.90	193.90±2.34
2/1 ratio of SACS	D	4.62 ±0.12	7.75±0.11	12.12±0.36	1429.86±17.76	201.77±3.57
	E	4.64±0.25	5.75±0.03	18.27±0.02	1436.09± 30.16	205.30± 8.01
	F	4.60±0.20	6.04±0.01	16.97±0.02	1422.67± 5.44	187.0± 2.73
9/1 ratio of SACS	G	2.70±0.04	1.30±0.01	8.03±0.01	976.88±12.22	70.95±2.32
	H	2.57±0.05	2.42±0.01	12.12±0.36	1109.86±1.05	169.77±3.57
	I	2.33±0.20	1.92±0.04	16.97±0.02	1188.67± 5.44	70.00± 2.73

Ca) increased the best in contents after application of solution at 500 ppm concentration of SACS at 2/1 ratio but in 9/1 ratio, all nutrients decreased than control leaves. Na also has decreased at all ratios and control. Zn increased significantly at 350 ppm of 2/1 ratio of SACS treated leaves and Fe, K, Ca increased significantly at 500 ppm of 2/1 ratio of SACS treated leaves. Fe Zn, K, Ca increased 21%, 1.21%, 53% respectively as compared to control. Nutrients of group 1 (K) and 2 (Fe, Zn, Ca) metals showed increase in contents after the SA/CS application because they do not take part in chelate formation.

Researcher revealed that, treatment with irradiated CS enhanced the activity of chitinase in treated plants and also improved the survival ratio and growth of the transferred plantlets acclimatized for 10-30 days under greenhouse conditions. [32]. Supplementation with optimum concentrations of irradiated CS resulted in a significant increase in the fresh biomass (68.1% for chrysanthemum, 48.5% for lisianthus, 53.6% for limonium and 26.4% for strawberry), shoot height (19.4% for chrysanthemum, 16.5% for lisianthus, 33.9% for limonium and 25.9% for strawberry) and root length (40.6% for chrysanthemum, 66.9% for lisianthus, 23.4% for limonium and 22.6% for strawberry [29-32]. SA and CS is an excellent chelator of many metal ions (e.g. Cu, Ca, Ni, Co, Pb, etc.). Thus it absorbs Fe and Ca ions by forming metal complex and retain on the leaf.

5.11 Heavy metal uptake on betel vines

The effect on heavy metal uptake by piper betel vine after 30 days due to applying irradiated SACS of different concentrations and ratios were established on table 4. After 30 days foliar spraying of SACS on betel vine Pb decreased only in the leaves of 2/1 ratio at 350 ppm than the control leaves. In the leaves of 2/1 ratio and 9/1 ratio at various concentration, Pb increases a little as compared to the the control leaves.

In the 2/1 ratio of 500 ppm and 750 ppm SACS treated specimen, Cr consumption decreases 43% and 67% respectively than control specimen. In all other ratio and concentrations Cr increases in treated specimen as compared to the control leaves. Permiissble amount of As and Hg consumption were found in the contol and treated leaves.

As and Hg showed concentration less than 0.1 and 0.3 mg/kg respectively. Due to spraying of various ratios of SACS on betel vines, Cd consumption in leaves showed more or less same results to the control. At higher concentration the polymer coil up, did not present at straight chain. So they show resistant to chelation to few metals. However, due to the 30 days foliar spraying of SACS on betel vines, Pd content has deceased to a significant level 31% in 2/1 (SACS) ratios of 350 ppm solution concentration.

Table 4: Effect of irradiated SACS on betel vines' heavy metal uptake after 30 days of foliar spray.

Treatment	ID	Heavy metal uptake (mg/kg)				
		Pb	**Cd**	**Cr**	**As**	**Hg**
Control	O	3.17±0.03	0.22±0.003	0.10±0.010	>0.1	>0.3
1/1 ratio	A	3.30±0.001	0.23±0.001	0.12±0.01	>0.1	>0.3
	B	3.30±0.03	0.23±0.001	0.12±0.05	>0.1	>0.3
SA/CS	C	3.31±0.15	0.22±0.001	0.13±0.015	>0.1	>0.3
2/1 ratio	D	2.43±0.09	0.23±0.004	0.12±0.005	>0.1	>0.3
	E	3.25±0.02	0.23±0.003	0.07±0.018	>0.1	>0.3
SA/CS	F	3.27±0.09	0.22±0.005	0.06±0.003	>0.1	>0.3
9/1 ratio	G	3.32±0.05	0.22±0.001	0.18±0.008	>0.1	>0.3
	H	3.33±.08	0.22±0.003	0.15±0.015	>0.1	>0.3
SA/CS	I	3.34±0.05	0.23±0.007	0.16±0.002	>0.1	>0.3

The treated soil also showed lower concentration of the minerals, which may be due to the translocation of them from the plant location.

5.12 Nutrients and heavy metals content in soil of betel vine garden

The effect on heavy metal consumption in soil of the tested betel vine garden were presented in table 5. It was observed in table 4 that the transition metals have shown significant decrease in soil close to the tested area of betel vine garden and Zn, Fe, Na, K and Ca showed also increase in this area.

Table 5: Effect of SACS on nutrient and heavy metals content in soil of betel vine garden after 30 days of foliar spray.

Parameter	Unit	Soil taken close to the tested area of SACS			Soil taken away from the tested area of SACS		
Pb	mg/kg	24.657	±	0.1726	31.4784	±	0.063
Cd	mg/kg	0.8946	±	0.0152	1.04928	±	0.0094
Cr	mg/kg	16.43	±	0.0986	18.581	±	0.0929
Zn	mg/kg	27.624	±	0.221	32.70256	±	0.0654
Fe	mg/kg	13245.00	±	66.224	26516.18	±	79.549
Ca	mg/kg	1070.9	±	1.0709	349.76	±	2.0986
Na	mg/kg	102.77	±	5.7552	43.0642	±	4.2634
K	mg/kg	948.3	±	3.7932	841.3914	±	4.207
As	mg/kg	1.091	±	0.024	2.14228	±	0.0578
Hg	mg/kg	<0.1			<0.1		

The treated soil also showed lower concentration of the minerals, which may be due to the translocation of them from the plant location.Soils were collected under the SACS treated betel vine and under the control plants.

After 30 days foliar spraying of SACS on betel vine Pb, Cd, Cr and As decreased in the soil of the treated area as regards 28%, 17% and 96% respectively than the soil of the control area. Hg consumption in the soil of the experimental betel vine garden and away from the betel vine garden is less than 0.1. Zn and Fe decreases 19% and 100% respectively in the soil of treated area than the soil of the control area. Ca, Na and K increases in the soil of the treated area as regards 206%, 137% and 13% respectively than the soil of the control area.

5.13 Disease on betel vine

The effect on reduction of disease due to 90 days foliar spraying of SACS of different concentrations and ratios on piper betel vine were establish on figure 7. Compared to control one, all ratio of SACS mixture showed potential action against disease. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration the disease decreased 90%, 86% and 83%% respectivelythan control. Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration the disease decreased 103%, 104% and 99% respectivelythan control . Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration, the disease decreased 78%, 75%, and 70% respectivelythan control .

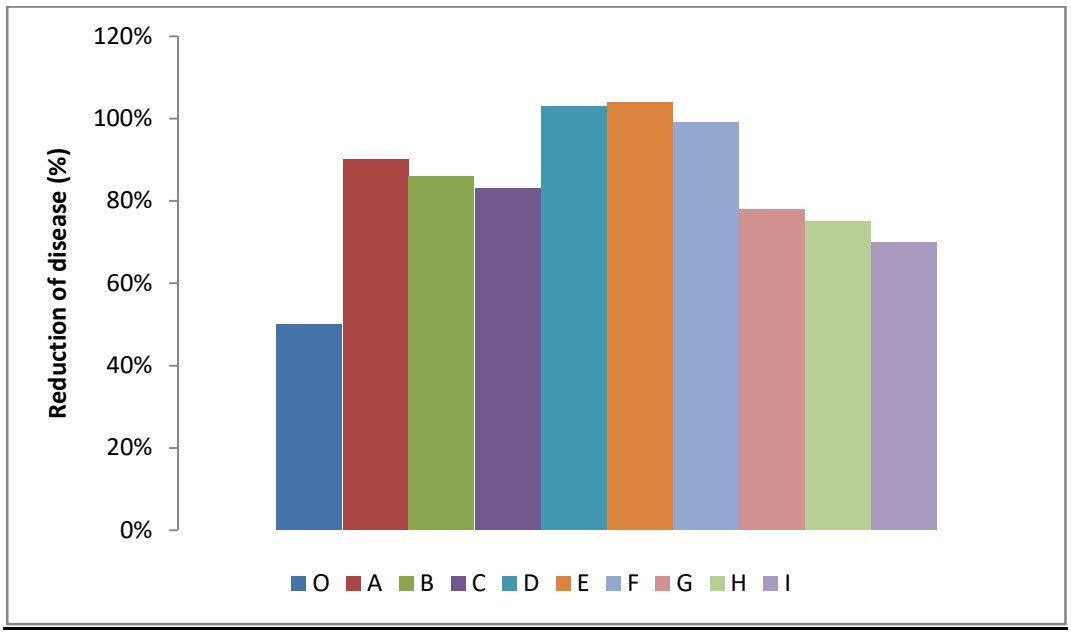


Figure7. Effect of irradiated SACS on betel vines' reduction of disease after 30 days of foliar spray.

From figure 7, reduction of disease decreases for the mixures of 50 kGy chitosan solution and 12kgy sodium alginate at different concentrations are ranked as follows:

Control >9/1 Ratios of SACS> 2/1 Raios of SACS>1/1 Ratios of SACS

From the figure 7 it is clear that, mixture of 2/1 ratios shows maximum antifungal activity in contrast with the other treatment and control plants.

Stimulation of growth of bifidiobacteria to resist infection of diseases for plants particularly oligoCS in agriculture as biotic elicitor to enhance defense response sagainst diseases [11] and suppression of heavy metals stress [12]. Different processing technologies have been applied to transfer natural polymers into the market able products.

5.14 Anti-fungal test

The betel vine plant farmers facing heavy loss due to leaf rot of betel vine (described details in chapter 1) caused by various Fungus was counted in various petri plates after incubation. Total numbers of fungi were calculated by colony counter in PDA media (described details in methodology section). It was observed that, the total fungal count for control, nonirradiated SA and nonirradiated CS was 462.60 x10^2, 88.02 x10^2 and 86.74 x 10^2 cfu/g respectively. Foliar spraying of 1/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration the fungal count decreased 28.70 x 10^2, 32.70 x 10^2 and 26.40 x 10^2 cfu/g respectively. Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration the fungal count decreased 22.90 x 10^2, 26.40 x 10^2 and 20.70 x 10^2 cfu/g respectively. Foliar spraying of 9/1 ratio of SACS on betel vine at 350 ppm , 500 ppm and 750 ppm concentration, the fungal count decreased 32.60 x 10^2, 37.70 x 10^2 and 38 x10^2 cfu/g respectively.

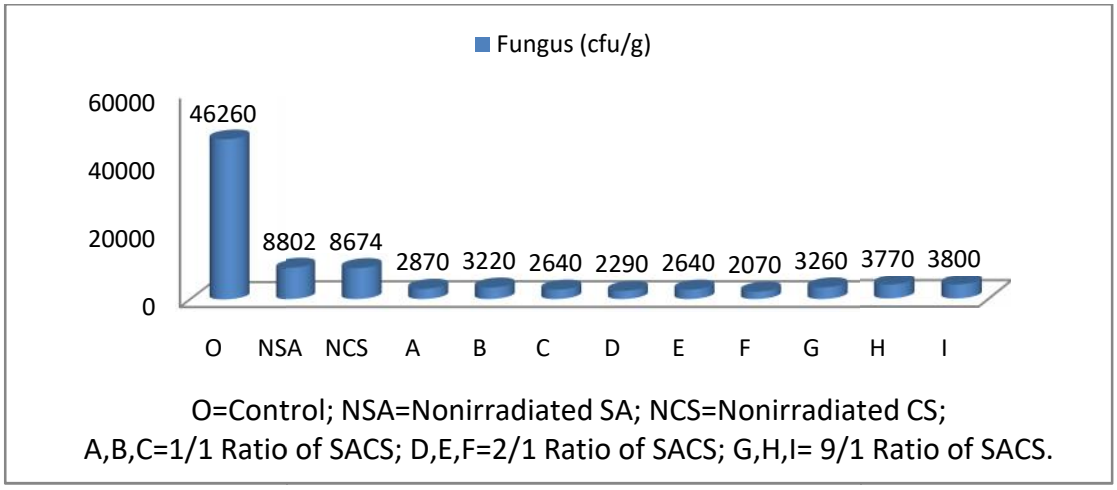


Figure 8. Anti-fungal activity of radiation processed sodium alginate and chitosan mixture (SACS) on betel vine plants

From figure 8, fungal count for the mixures of 50 kGy chitosan solution and 12kgy sodium alginate at different concentrations are ranked as follows:

Control > NSA>NCS> 2/1 Ratios of SACS> 1/1 Raios of SACS>9/1 Ratios of SACS

From the figure it is clear that, mixture of 2/1 ratios shows maximum antifungal activity in contrast with the other treatment and control plants.

For control the total fugal count was 462.6×10^2, which is reduced to 26.4×10^2 , $22.9x10^2$ and $32.6\ x10^2$ when plants are treated with the mixure of chitosan and alginate solution at a ratio of 1/1 , 2/1 ratio and 9/1 ratio respectively. If we consider with NCS and NSA we found that, with all ratios, total fungal count was decreased significantly.

CS, SA or carrageenan, a high molecular natural polymer,nontoxic,bioactive agent has become a useful appreciated compounds due to its bio-fertilizer, promotion of germination and shoot elongation,[2] as a growth stimulator on growth and yield of rice, wheat, maize, black pepper, bean, cabbage, peanut, soybean, tomato, cotton, strawberry[3-5] in orchid tissue culture,[6] fungicidal effects or elicitation of defense mechanisms in plant tissues[7-10,22].

5.15 Shelf life of SACS treated betel leaves

After 30 days foliar spraying of 1/1, 2/1 and 9/1 ratios of SACS at 350 ppm, 500 ppm and 750 ppm concentration on betel vine, matured leaves were collected and then weighed. These leaves were kept separately in an air tight bag and preserved in a refrigerator for 30 days. After preservation of one month it was observed that, control and all treated betel leaves were almost 70-100% damaged, only foliar spraying of 9/1 ratio of 500 ppm SACS treated betel leaves achieved their shelf life 60%.

CS is one of the most important marine polysaccharide has many peculiar biological activity ssuch as immunity, nor cholesterol and antibacterial [20, 21]. Degradation of CS is usually used, turning CS in to one with low molecular weight which exhibits good water solubility. The water-soluble CS with low molecular weight has some special biological, chemical and physical properties which are different from that of the ordinary CS such as antibacterial activity, [22] antifungal activity [23] and antitumor activity [24]. The functionalized polymers revolutionized the agricultural, horticultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients etc.

Except this, blemished and damaged percentage was also showed for the various treatments of SACS are ranked as follows:

- Weight: A>D>G>I>B>F>E>C>H>O
- Damaged control: H>G,I,O

Sample ID	**Total wt. in g**	**Damaged percentage (%)**	**Shelf life of SACS treated betel leaves**		
			2/1 ratio of 350 ppm SACS (G)	**2/1 ratio of 500 ppm SACS (H)**	**2/1 ratio of 750 ppm SACS (I)**
O	20.4	100%			
G	33.12	100%			
H	34.4	40%			
I	30.75	100%			

Figure 9. Shelf life of CS and SA mixture (SACS) treated betel leaves

CS is one of the most important marine polysaccharide has many peculiar biological activity such as immunity, nor cholesterol and antibacterial [20, 21]. Degradation of CS is usually used, turning CS in to one with low molecular weight which exhibits good water solubility. The water-soluble CS with low molecular weight has some special biological, chemical and physical properties which are different from that of the ordinary CS such as antibacterial activity, [22] antifungal activity [23] and antitumor activity [24].

The functionalized polymers revolutionized the agricultural, horticultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients etc.

5.16 Sensory evaluation test

Sensory evaluation test were conducted by 70 persons by eating treated and untreated betel leaves. Except this, by survey it distinguished that most of the peoples didn't know why they chew this leaves. Only for eating this leaves, they consider nothing. Chitosan treated betel leaves acceptance the most.

After 30 days foliar spray of nonirradiated, irradiated CS and various ratios of their mixtures on piper betel vine, the sensory evaluation test was conducted by ranking procedures. Samples were served with no giving any information of the differences between samples. Indirect samples, interval between tasting, orders of presentation

etc. were also followed. Five samples for each concentration were presented at the same time and Rank positions 1 to 10 were given in order of increasing the degree of some attributes e.g. color, texture, appearance and taste. Rank positions (0-10) are taken for the average value where '0' was the lowest value and '10' was the highest value. Differences between totals are tested for significance using Rank-Sum Difference tables. The leaves obtained from the sample treated at various ratio of SACS, all ratios were passed the test scoring below 5 out of 10, where the control sample in acceptance of 5 presented in table 6.

Table 6: The sensory evaluation test of various ratios of irradiated SACS treated piper betel leaves (by Ranking Procedures)

Person	Specimen	Color	Texture	Appearance	Taste
70	Control	4± 0.23	5 ± 0.16	5 ± 0.05	5 ± 0.08
	1/1 Ratios	2± 0.04	4±0.32	2 ± 0.14	3± 0.7
	2/1 Ratios	3 ± 0.04	3 ± 0.184	2 ± 0.5	3 ± 0.22
	9/1 ratios	3 ± 0.05	3 ± 0.31	3 ± 0.32	2 ± 0.12

5.17 TG of SACS treated betel leaves

Table 7 shows the weight loss percentage of irradiated SACS treated piper betel leaves at 30 to 600°C temperatures. There is no significant change in weight variation as observed from TGA analysis of control and SACS treated sample.

The cellulosic portion of the plants has been increased after the treatment applied.This is concordant with the result obtained from the TGA value. The weight retainig is obtained a little lower for the treated plants than that of the control after applying upto 600°C temperature to them. This confirms the enhanced growth of the plants by fertilizer like action of SACS mixture. Similar results were also obtained fro SA and CS when applied separately.

Table 7: TG analysis of irradiated SACS treated piper betel leaves

Sample ID	Weight retain (%) at various temperatures (°C)						
	0-25°C	100-110°C	200-250°C	300-350°C	350-430°C	500-550°C	550-600°C
Control (O)	100%	94%	89%	66%	52%	24%	20%
500ppm, 2/1 ratio of SACS treated betel leaves	100%	92%	85%	62%	53%	24%	18%

5.18 Scanning electron microscopy of pure and irradiated SACS treated betel leaves

The SEM images of the control (figure 10a) and various concentration of 2/1 ratio of irradiated SACS (figure 10 b, c, d) are shown in figure 8. There have no gignificant effect on SEM image of betel leaves after the 30 days foliar spraying of SACS.

Figure 10a (Control)	Figure 10b (350 ppm, 1/1 ratio of SACS)

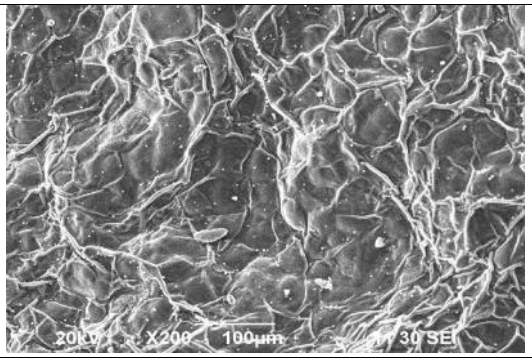
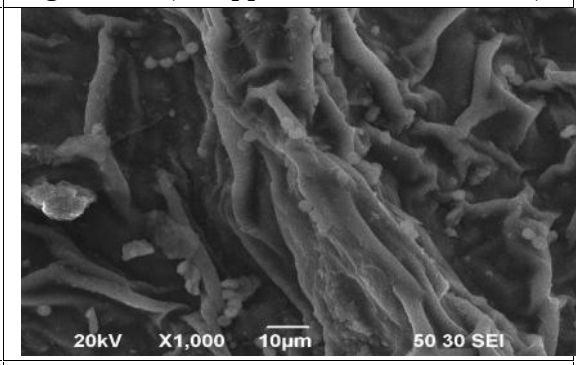

	

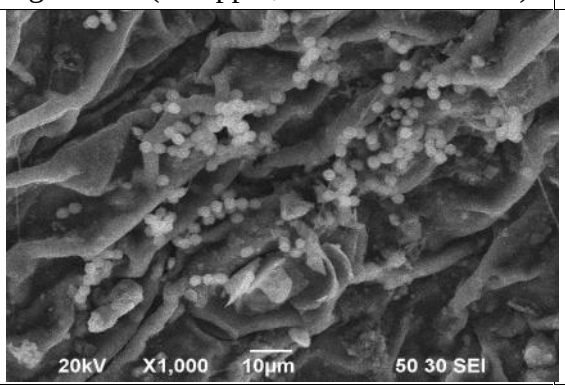
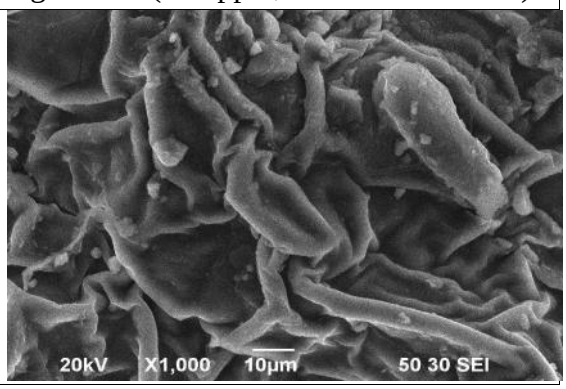
Figure 10b (500 ppm, 2/1 ratio of SACS)	Figure 10b (750 ppm, 2/1 ratio of SACS)
	

Figure 10. SEM photograph of various concentrations of 2/1 ratios of SACS on betel leaves

5.19 Observation

- Foliar spraying of 2/1 ratio of SACS at on betel vine, 500 ppm shows the best plant height increasement at 57% whereas the control increases 36%.
- In 2/1 ratio of SACS on betel vine at 350 ppm concentration increased number of leaves found at 147% whereas control increases 24%.
- Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm concentration increased best shoot growth found 300% than control .
- Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm concentration increase the best vine's thickness as regards 140% where as control plants increased this value of 41%.
- Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm concentration increase the best area of leaves as regards 127%, where as control plants increased this value of 27%.
- Foliar spraying of 2/1 ratio of SACS on betel vine at 350 ppm concentration increase the best wet weight value of leaves as regards 5.39% than control.
- Foliar spraying of 1/1 ratio of SACS on betel vine at 500 ppm concentration increase the best roots' value of 100% than control.
- After 30 days foliar spraying of SACS on betel vine Pb decreased the in the leaves of 1/1 ratio at 350ppm, 500ppm, and 750 ppm concentration as regards 220% than the control leaves.
- Cd consumption is less than 0.01 mg/kg in the 350 ppm 1/1 ratio of SACS treated leaves.In the leaves of 2/1 ratio at 350 ppm concentration, Cd decreased 60 %, than the control leaves.
- Permiissble amount of Cr, As ans Hg consumption in the contol and treated leaves were determined.
- Most of the nutrients analyzed (e.g. Fe, Zn, Na) showed contents after application of solution at 350 ppm concentration of SACS at 2/1 ratio but K, has increased significantly at 500 ppm of 2/1 ratio. Ca has increased in 750 ppm of 1/1 ratio. Nutrients of group 1 and 2 metals showed increase in contents after the CS application because they do not take part in chelate formation.
- After 30 days foliar spraying of SACS on betel vine Pb, Cd, Cr and As decreased in the soil of the treated area as regards 28%, 17% and 96% respectively than the soil of the control area. Hg consumption in the experimental betel vine garden is less than 0.1.
- Zn decreases 19% and Fe were also decreases 100% in the soil of treated area than the soil of the control area.
- Ca, Na and K increases in the soil of the treated area as regards 206%, 137%, and 13 % respectively than the soil of the control area.
- Foliar spraying of 2/1 ratio of SACS on betel vine at 500 ppm concentration the disease decreased at 104% than control.
- It was observed that, the total fungal count decreased the best after the foliar spraying of 2/1 ratio of 750 ppm SACS on betel vine than control,

- Foliar spraying of 2/1 ratio of SACS on betel vine at 500 ppm concentration treated betel leaves achieved their shelf life as regards 40%.
- There have no significant effect on SEM image due to concentration variation of various ratios of SACS on betel vine.
- The weight retainig is obtained a little lower for the treated plants than that of the control after applying upto 600°C temperature to them.
- The leaves obtained from the sample treated at various ratio of SACS, all ratios were passed the test scoring below 5 out of 10, where the control sample in acceptance of 5.It is lest testied than SA ,CS and control also.
- This confirms the enhanced growth of the plants by fertilizer like action of SACS mixture. A well-coordinated effort by the farmers, traders, scientists, administrators and policy makers is required to be initiated to boost up the national economy through proper exploitation of this green gold “betel vine”.

5.20 References

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Chapter 6: Conclusion

Chapter 6: Conclusion

6.1 Economic aspects of SA and CS

The cultivation of betel vine is highly specialized and need intensive care. However, the table 1 and table 2 in this paper demonstrate that, gamma irradiated SA as plant growth promoter on betel vine is economically more profitable than the CS treated and as well as control sample. Total cultivation cost of 5000 betel vines by irradiated SA and CS in 6 months is 5, 64,195 BDT/= and 566,195 BDT/= respectively. On the contrary, total cultivation cost of control betel vine is 5,49,340 BDT/=which could be produced only 1500 bira leaves in 6 months and selling price of that produced 1500 leaves is 1,20,000 BDT/= which is the much less than treated one. Irradiated SA and CS treated betel vines produces 3600 bira and 3300 bira leaves in 6 months respectively. Selling price of these 3600 bira and 3300 bira betel leaves is 4,32,000 BDT/= and 3,96,000 BDT/=. Moreover, the effect of gamma irradiated SA and CS on betel vine shows larger leaf size which is actually leaves of export quality.

Table 1: Income by SA treated betel gardening in 6 months

	Matured leaves/week	**Production in 6 months for 5000 vines**	**Income/selling price** (BDT)
Control	1 leave /vine/wks. (10 leaves in 10 plants)	5000 pcs./week × 4wks. ×6 xonths=1,20,000pcs. leaves = **1500 bira**	1500 bira × 80 BDT (small size)= **1,20,000 BDT**/=
SA treated	2.4 leaves/vine/wks. (24 leaves in 10 plants)	12,000pcs./week.×4wk. ×6months=2,88,000 pcs. leaves=**3600 bira**	3600 bira ×120 BDT (big size)= **4,32,000 BDT**/=
CS	2.2 leaves/vine/wks. (22 leaves in 10 plants)	11000pcs×week×4wk. × 6 months=264000pcs= **3300 bira**	3300 bira × 120 BDT (B(big size)=**3,96,000 BDT/=**

Table 2: Betel vine plant cultivation cost (price is iin BDT.).

Cost (in various purposes)	**Cultivation cost**	**Control**	**SA treated**	**CS treated**
Price of land (10 decimal)	5,00,000 BDT/=	5,00,000/=	5,00,000/=	
Price of betel vine	5,000 plants × 3 BDT = 15,000 BDT/=	15,000/=	15,000/=	
Labor cost	**Structure making**: 3 persons × 90 days × 400 BDT = 15,000 BDT/=	15,000/= 2,800/=	15,000/= 2,800/=	
	First day of plantation: 7 persons × 400 BDT = 2,800 BDT/=	- -	- -	- -
	Family labor: 3 persons (at no cost labor) [Profit by owner's self labor]	-	-	-
Soil processing cost (before plantation)	**PSP** (Black fertilizer): 50 kg × 40 BDT = 2,000 BDT/=	2,000/=	-	-
	Muriate of Potash (MOP) : 50 kg × 18 BDT =900 BDT/=	900/=	-	-
	Urea (White fertilizer) : 10 kg × 20 BDT = 300 BDT/=	300/=	-	-
	Gypsum (Tula fertilizer : 50 kg × 10 BDT = 500 BDT/=	500/=	-	-
	Salt required: 20 kg × 20 BDT = 400 BDT/=	400/=	-	-
	Cow dung: 20 bags × 50 kg × 200 BDT = 2,000 BDT/=	2,000/=	2,000/=	2,000/=
Total	**Vitamin**: 5 packet × 5 kg × 120 BDT = 3000 BDT/=	3,000/=	-	-

fertilizer cost in 6 months	**Epitaph** (for pest/disease control): 12 bottles (200 ml) × 120 BDT = 1440 BDT/=	1,440/=	-	-
Basket price	12 × 500 BDT = 6000 BDT/=	6,000/=	6000/=	6000/=
Cost of SA and CS for be Plants(5000)	**SA powder cost for 50 plants** 33.95 BDT/= So, for 5000 plants cost will be 3395BDT/=	-	3,395/=	-
	C**S powder cost for 50 plants**	-	-	5000/=
Radiation cost	12 kGy dose for 200 ml SA and CS solution	-	20,000/=	22000/=
Total cost	**5000 number of betel vine in 6 months**	**5,49,340/=**	**5,64,195/=**	**5,66,195/=**

6.2 Conclusion

This study was aimed to develop a natural polymer based plant growth promoter in order to reduce chemical fertilizer utilization in agriculture. Chitosan and Sodium alginate after irradiation have been applied as plant growth promoter on selective crops; Tomato, Cabbage, Cauliflower, Spinach, Mung Dal, Pumpkin, Jute, Eggplant, Green chilli Red chilli and Betel vines. Several parameters of their growth have been investigated like Plant's heights, size of the plants, number of leaves, leaves areas, number of stems, length of their roots, fruits size, thicknesses of the vine and weights. Piper Betel vine has been taken in a special consideration among them, because it as a cash crop and all over the world other than Bangladesh, there are a few countries which cultivate betel vines. For betel vines farming, nutrient and heavy metal contents in the betel leaves and heavy metal contents in the soil of cultivation was observed. Disease control action, antifungal nature and shelf life effect were observed higher for treated betel leaves.

1. Radiation causes chain scission of SA and Chitosan and their molecular weight reduced to 5000 Da and 90000 for SA and CS respectively. Chitosan was obtained with 78% of DD.
2. Spinach, Pumpkin, Mung dal, Eggplant, Tomato and Jute showed increase of plants' height, number of leaves, leaves area, shoot growth and root length of them after application of SA and CS. These polymers act as fertilizers on the growth and productivity of the plants and showed increase in several plant parameters compared to control ones. However, it isobserved that the growth promotion activity of both SA and CS varies on different parts of the plants at different rate.

3. Betel vine showed increases of plant height, number of leaves, leaves area, number of shoot, root length & thickness, number of stems from 10% to 288 % in different cases after application of SA and 50- 200% increase after CS application.
4. CS showed better chelation activity than SA for nutrient uptake as it contains amine group in it. Significant amount of Fe and Ca uptake was observed on betel vine after the 750 ppm irradiated SA treatment and 350 ppm CS application. Among various concentration of the polymers applied, it was observed that lower concentration facilitated a better penetration and activity. At higher concentration the polymer coil up, did not present at straight chain. So they showed resistant to chelation.
5. Sensory evaluation test ranked 10 out of 10 for chitosan applied sample whereas the control ranked 3 only.
6. CS application increased betel leaves' shelf life and reduces diseases attack. Shelf life of control leaves are 10 days and that of CS treated leaves were observed upto 1 month at 8°C in refrigeration. Significant reduction of diseases was observed on betel vine after the chitosan application.
7. From the mixture of SA and CS, there was no significant synergistic effect observed on betel vines due to variation of ratios of SA and CS.
8. TGA and SEM analyses confirmed no retaining of the polymers on the leaves.
9. From economic estimation, it has been found that the income from selling price of betel leaves can increase around 300%.
10. This thesis work fully investigated the plant parameters especially on betel vines after application of SA and CS as biofertilizers. The polymers are derived from waste natural sources and they not only work as plant growth promoters rather they also improve nearby soil and environmental contamination. Commercialization of SA and CS as biofertilizers needs a well-coordinated effort by the farmers, traders, scientists, administrators and policy makers to include a modern and innovative approach inour agricultureand keep better environment.
11. The effect of gamma irradiated SA and CS on betel vine shows larger leaf size which is actually leaves of export quality.

List of publications

1. **Journal papers (Published paper)**
   - Ferdous Aktar, Papia Haque, Mubarak Ahmad Khan, Ahmad Ismail Mustafa (2017). Effect of Gamma Irradiated Sodium Alginate on Selective Crops as Plant Growth Promoter and Bug control action, "***International Journal of Nanotechnology in Medicine & Engineering"***. 2:10,163-172.

2. **Participation on Congress (Published abstract)**
   - Ferdous Aktar, Papia Haque, Mubarak Ahmad Khan, Ahmad Ismail Mustafa, (2016), A Study of Plant Growth Promoting and Anti-fungal Activity of Radiation Processed Sodium Alginate on Betel Vine Plants, ***"16th Asian Chemical Congress"***, March 16-19, Dhaka, Bangladesh

   - Ferdous Aktar, Papia Haque, Mubarak Ahmad Khan, Ahmad Ismail Mustafa (2018). Effect of Gamma Irradiated Chitosan on Betel Vine Plants as Growth Promoter within Assessment of Yield, Disease Control Action and Nutrient Content. ***'10th (AFOB) Asia federation of Biotechnology Regional Symposium (ARS 2018)',*** Dhaka, Bangladesh, January 27-29, 2018.

3. **Submitted paper**
   - Ferdous Aktar, Papia Haque, Jahid M. M.Islam, Mubarak Ahmad Khan, Ahmad Ismail Mustafa. Revolutionize of the agricultural by the foliar application of gamma irradiated Chitosan (CS): for the molecular treatment of diseases and enhancing the ability of plants to absorb nutrients on Betel vine, ***"The International Journal of Biotechnology and Bioengineering (IJBB).*** BioCore group, ISSN-2475-3432, Virginia, USA (Accepted).
   - Ferdous Aktar, Papia Haque, Romana Islam, Jahid M M Islam, Tasrina Rabia Chowdhury, Mubarak Ahmad Khan, Ahmad Ismail Mustafa, Plant Growth Promotion and Nutrient Uptake of Radiation Processed Sodium Alginate on Betel Vine Plants, ***'The Journal of Applied Science and Engineering"***, The Faculty of Engineering and Technology, University of Dhaka, Bangladesh, May 2016 (No. 188).