APPLICATIONS OF RADIATION-PROCESSED CHITOSAN IN AGRICULTURE

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Abstract:

Chitosan is a natural biopolymer composed of randomly distributed β-(1,4)-linked D-glucosamine (2-amino-2-deoxy-D-glucopyranose) and N-acetyl-D-glucosamine (2-acetamido-2-deoxyD-glycopyranose) units. In nature, crustacean shell biomass, one of the major waste-product of marine/sea-food industry, is the source of chitosan production. The application of chitosan affects various physiological processes in plants including cell division, cell elongation, nutrient uptake, enzymatic activation and protein synthesis, resulting in improved growth and yield. In addition, chitosan mediated biocontrol properties for crop protection may be attributed to its elicitor response towards various plant pathogens. Apart from agriculture, chitosan also has many diverse applications in the fields of medicine, pharmaceutical, and food; however, its true potential is still limited due to its low-solubility. With this background, the present chapter highlights the potential of gamma-radiation for depolymerizing chitosan, with the aim of being used as a plant-growth bioregulator for ensuring sustainable agriculture. The bio-efficacy of gamma-irradiated chitosan has been demonstrated in multiple crops, including horticultural plants. Considering this, an indigenous technology named “Anu-Chaitanya” has been developed using gamma-irradiated chitosan that can be seen as a typical example of “wealth-from-waste” and “peaceful use of radiation”.

Key words: Benefit:cost ratio, chitosan, crop yield, depolymerization, elicitor, plant signalling.

INTRODUCTION:

Chitosan is a linear mucopolysaccharide and, in its natural form, composed of randomly distributed β-(1,4)-linked D-glucosamine (GLcN; 2-amino-2-deoxy-D-glucopyranose) and N-acetyl-D-glucosamine (GlcNAc; 2-acetamido-2-deoxyD-glycopyranose) units (Fig. 1A). A substance is categorized as chitosan if the concentration of these acetyl-glucosamines, termed as degree of acetylation (DA), is lower than 50% and chitin if DA is 50% or more (typically ~100%)[1]. Apart from DA, degree of polymerization (DP) is also an important property-defining factor correlating well with chain length and molecular weight (MW) in kD. The DP practically used for short and precisely defined chain length for example, DP8 is an octamer. For larger oligomers and polymers, average MW in kDa may be used to define the DP-variation in certain range[2]. After cellulose, chitin (β-1,4-N-acetyl-D-glucosamine) emerges out as second most copious natural biopolymer in the biosphere. The chitin, as an enriched source of carbon and nitrogen, has been shown to be important for maintaining the marine ecosystem within the ocean. Besides, owing to their nontoxic, biocompatible, and biodegradable polymers, both chitin as well as chitosan have enormous applications in diverse fields.

The industrial production of chitosan and chitin utilises exoskeleton of marine crustaceans, crab, shrimp, squid bone plates, and the fungi's cell walls as primary source[2]. Nevertheless, most of the chitin/chitosan is derived from crustacean shell biomass, which has abundant inexhaustible resource and support unconventional waste management strategy. The crustacean shell is a natural composite material, comprised of structural proteins (30–40%), $CaCO_3$ (30–50%) and various forms of calcium phosphate, and chitin (20–30%) along with trivial amount of lipids and pigments[3, 4]. The downstream processing and valorization of chitin begin with its extraction, which involves washing and grinding, demineralization (treatment with HCl or other acids such as HNO_3, H_2SO_4, CH_3COOH, or HCOOH), deproteination (mixing with NaOH at temperatures up to 160°C) and finally removal of pigments and lipids using $KMnO_2$. Although, NaOH is industrially adopted for deproteination but other alkalis such as KOH, Na_2CO_3, K_2CO_3, $NaHCO_3$, $CaHSO_3$, $Ca(OH)_2$, Na_2S, and Na_3PO_4 have also been used successfully[5]. All these processing resulted in random breakdown of the chitin backbone and random deacetylation, resulting in mixture of chitosan-oligomers (COS) having undefined DP and DA. Alternatively, biological processing of shrimp waste may offer an alternate eco-friendly approach which can diminish the environmental burden of the acid/alkali residues as well as minimise the unwanted changes in the chitin structure. One such method, utilise naturally produced lactic acid for demineralization and proteases for deproteination. Similarly, co-fermentation of shrimp waste with proteolytic and lactic acid bacteria was also reported to facilitate the better-defined chitosans production under relatively milder treatment conditions (Fig. 1B).

DIVERSE APPLICATIONS OF CHITOSAN:

The change in the solubility was one the important property associated with chitin-to-chitosan conversion. In contrast to chitin (insoluble), chitosan can be solubilised in acidic aqueous solutions. Therefore, chitosan emerges out as a versatile bioactive substance having superior matrix and functional properties. Being a promising bioactive material, chitosan and its derivatives have potential to interact with other bio-molecules and mediate broad-range biological effects, including antimicrobial, anti-inflammatory and anti-cancer activities, antioxidant and chelating capacities, as well as fat-binding, film-forming capability, leading to its potential industrial applications (Fig. 1C). Chitosan is also used as a preservative, dietary supplement, nutrient encapsulation and packaging additive in the food industries[6]. Similarly, cosmetic industry employed the antioxidant and antibacterial property of chitosan for developing skin protection products, toothpaste and mouthwash, and film-forming property for manufacturing shampoos and lotions[7]. The wastewater treatment systems also utilise the fat-binding and chelating properties of chitosan for the removal of fats, dyes and heavy metals[8, 9]. Since chitosan is non-toxic, biocompatible and biodegradable, have tremendous application as antimicrobial and anti-inflammatory agent in medical applications[10] like in wound dressings, as non-viral vector for gene therapy and as a drug delivery agent in cancer treatment[11, 12].

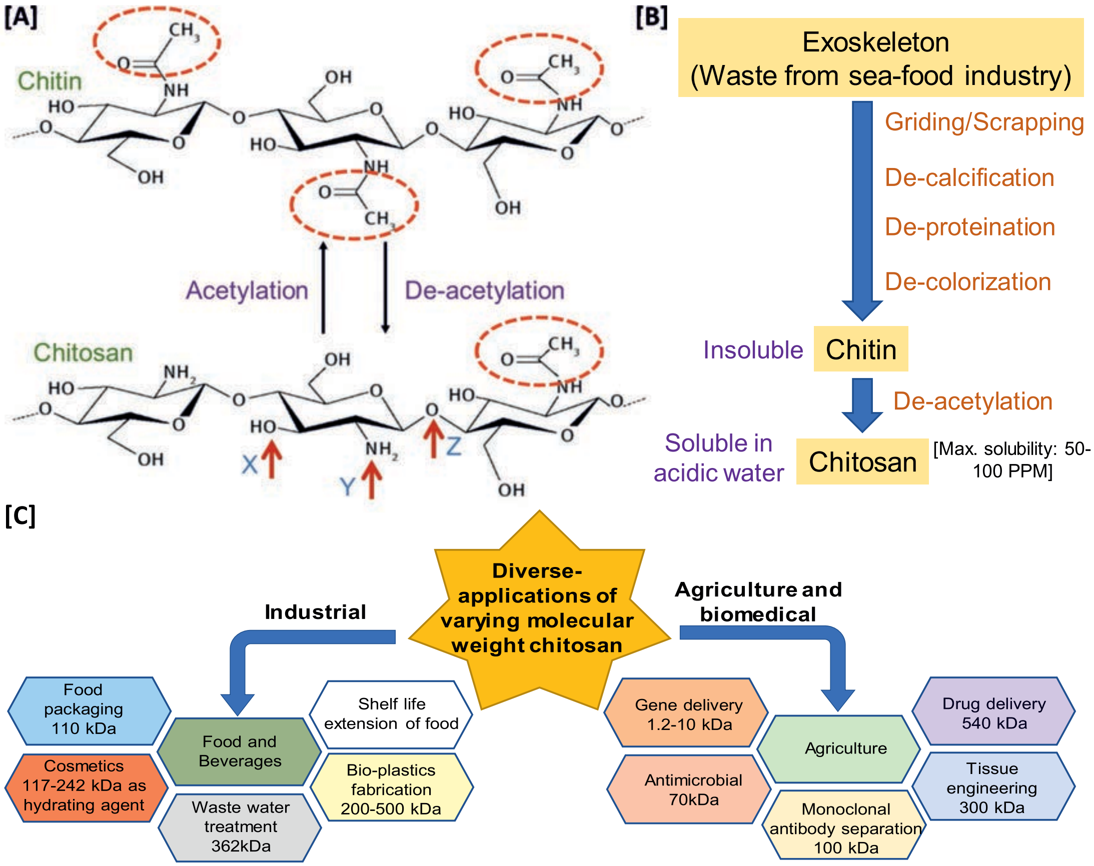

Fig. 1: Chitin and chitosan: A natural biopolymer. [A] Structure of chitin and chitosans. The red arrows in chitosan highlight different functional groups that can be modified, including sulfonylation/alkanoylation (X), chemical crosslinking/metal coordination (Y) and glycosidic bond cleavage degradation (Z). [B] Work-flow for the chitin-to-chitosan conversion. [C] Applications of chitosan in diverse fields.

The bioactive properties of COS are found to be superior relative to longer chitosan polymer, owing to high solubility and capability to bind nucleic acids and certain drugs in particular. Apart from DP and DA, the pattern of acetylation (PA) is another important factor which governs the general properties of chitosans such as solubility and type of chemical interactions. The pattern of acetylation (PA) is depicted as the sequence of GlcN and GlcNAc units across molecular backbone[13]. For instance, higher molecular weight chitosan (28–1671 kDa) has been shown to have stronger antimicrobial effects relative to COS with a lower molecular weight (1–22 kDa)[14]. However, COS within the DP range 2–15 exhibit higher antifungal activity than chitosan (MW 2000 kDa)[15]. Similarly, short and fully deacetylated chains (DP1–DP8, DA = 0%) showed superior antitumor activity as compared to that of chitosan (MW ~1900 kDa, DA = 1.5%)[16]. In agro-industry, chitosan is globally used to safeguard plants from fungi, bacteria, and viruses, as a plant growth regulator, and as a fertilizer additive [17,18,19,20]. Taken together, the well-grounded chitosan/COS production of defined properties is indispensable to ensure biological and physiological efficacy. Further, since an infinite combination of molecules, could be synthesised by systematically controlling variables like DA, DP and PA, the production of altogether new COS might allow the discovery of noble modes of action associated with potential new biological and physiological functionalities. This is clearly seen in terms of continuously increasing number of publications in NCBI-Pubmed (https://pubmed.ncbi.nlm.nih.gov/), in the last 23 years (Fig. 2).

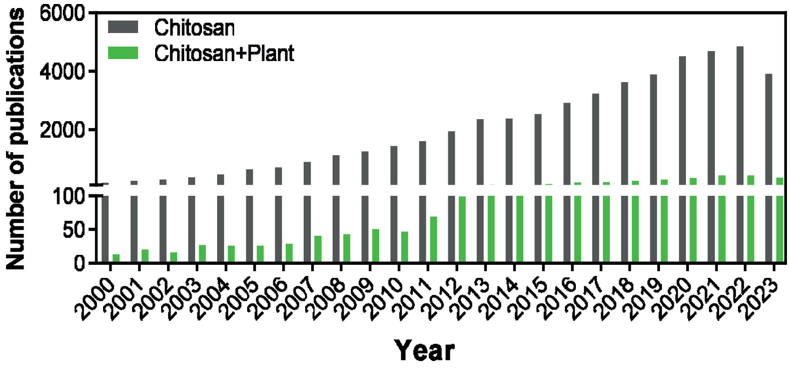

Fig. 2: Total number of publications on chitosan. Two independent keywords (chitosan and chitosan+plant) were used to search the number of publications in NCBI-Pubmed over the last 23 years. The data represent the literature search on 29th September, 2023.

Radiation-Induced Depolymerization of Chitosan for Agriculture Use:

The multiple applications of chitosan have been demonstrated in diverse fields; however, its actual potential is still not realized because of its low solubility at physiological pH. Chitosan is classified into high- (HMW; (700 kDa), medium- (MMW; 150–700 kDa) and low- (LMW; 150 kDa) molecular weight and each of these categories have their own specific applications (Fig. 1C). Currently, various approaches have been tested to obtain LMW-chitosan derivatives, with the aim to enhance their biological potential, over the native HMW-chitosan. Although, LMW-chitosan can be effectively produced by several ways, these processes have some demerits like require longer treatment time, have low productivity and selectivity, high processing cost (enzymatic method) and production of toxic by-products. Towards this endeavour, the application of gamma radiation has emerged as a suitable alternative to chemical/enzymatic treatment, for reducing the size of chitosan that has been demonstrated to have better bio-efficacy in terms of growth enhancement and/or providing stress tolerance in different crops[17-33]. So far, various doses ranging from 25-1000 kGy have been tested for depolymerizing the chitosan (Fig. 3); however, a suitable dose can be selected on the basis of sample homogeneity of the chitosan sample post-irradiation, in terms of particle size. Apart from the size reduction, other physical properties of gamma irradiated-chitosan (GIC) also gets changed. The most obvious and evident changes were seen in terms of viscosity and turbidity, which were decreased by 94 and 96%, respectively, in response to 100 kGy of gamma-radiation, compared with those of unirradiated control (Table-1). The average particle size of chitosan ranged from 4.2-6.5 µM; however, post-irradiation, it was reduced to 30-100 nM, with ~99% homogeneity. Owing to this, GIC can be seen as radiation-converted chitosan nanoparticles (ChNPs), instead of usual biological or chemical methods, although their relative comparison in terms of bio-efficacy is yet to be done. Unlike the specific-length chitosan having a fixed DA/DP, the radiation-processed chitosan contains multiple fragments with variable combinations of length as well as deacetylation. Due to this, GIC provides a unique advantage of inducing broad-range responses that is essential for optimizing plant-growth under diverse environmental conditions. Besides gamma, electron-beam (e-beam) radiation has also been used for depolymerization of chitosan. In combination with H_2O_2, synergistic effect was seen, which is associated with reactive hydroxyl radical formation due to the presence of e-beam[34].

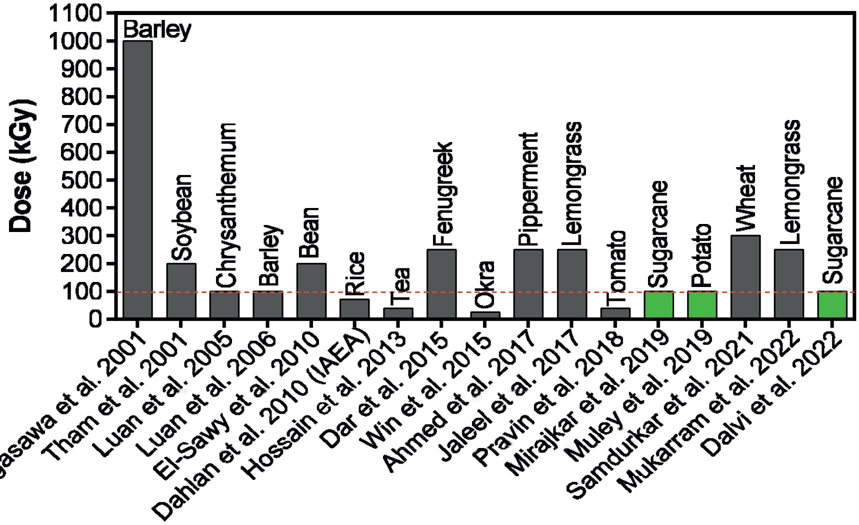

Fig. 3: Range of doses used for radiation-induced depolymerization of chitosan. A range of doses have been used for radiation-induced depolymerization of chitosan, to be tested in different crops[17-33]. The green-bars represent the studies related to the development of Anu-Chaitanya technology.

Table-1: Comparison of physical property between chitosan and gamma-irradiated chitosan (GIC; 100 kGy), at a fixed concentration of 5000 PPM.

Parameters	**Chitosan**	**GIC**
pH	3.17	3.17
Electrical conductivity	60	90
TDS	30	40
Viscosity (Centi poise)	250	15
Turbidity (OD600)	0.238	0.0086

Development of Anu-Chaitanya Technology for Sustainable Crop Production:

Chitosan application is known to stimulate plant growth by regulating various physiological processes like cell division, cell elongation nutrient uptake, enzymatic activation and protein synthesis leading to improved growth and yield. Although most of the beneficial effects of chitosan are attributed to the modulation of redox and phytohormone signalling[35, 36]; it is known to contain several other metabolites having diverse action. Chitosan also acts as an elicitor to trigger activation of defence system towards various plant pathogens through altered multiple metabolic pathways[35]. Owing to the positive impact of radiation-treatment on increasing the solubility, the bio-efficacy of GIC was evaluated to develop a versatile plant-growth promoting bioregulator. On the basis of screening, 100 kGy was selected as a suitable radiation dose for getting a uniform reduction of the particle size from the micro-to-nano range (Fig. 4A). The antioxidant capacity and plant growth promoting property of chitosan was found to be amplified after gamma-irradiation[29, 30, 33]. Initially, the replicated field trials were performed on sugarcane at Vasantdada Sugar Institute (Pune, Maharashtra). Sugarcane (*Saccharum officinarum* Variety Co86032) setts were treated with GIC (50 PPM) for 30 min, and subsequently, three foliar sprays were given at 30, 60, and 90 days after plantation. The agronomic parameters like cane yield was increased by 16% in GIC-treated plants (145 t/ha) than those of the water-sprayed control (125 t/ha). In addition, commercial cane sugar (CCS) was increased by 21% in GIC-treated plants (20.1 t/ha) than those of control (16.5 t/ha). The benefit:cost (B:C) ratio of using GIC was 1.65, with highest net profit of Rs. 66,352/-per hectare to the farmer. Inspired from these results, the positive impact of GIC treatment was further demonstrated in different crops including soybean, chickpea and ginger (Fig. 4B). In addition to crops, the foliar application of GIC was also found to be effective on horticulture plants, including *Tagetes erecta*, commonly known as marigold. It is a commercially important flower having diverse use in home gardening, professional landscaping, social and religious activities along with industrial application for extracting essential oil and active drug ingredients. The foliar application of GIC increased the flower number and flower diameter by 36.9 % and 27%, respectively, compared with those of the water-spray control (Fig. 4B). Thus, the GIC application was found to be versatile to be used for multiple crops including horticultural plants. Further, the GIC-based formulation has also been found to be compatible with other agronomic practices including intercropping and fertigation.

With this background, a formulation named “Anu-Chaitanya” was developed, which is purely indigenous in nature. One Anu-Chaitanya kit (20,000 PPM, 1 litre) is sufficient for two foliar applications in one-hectare area, costing approximately Rs. 1000/-, including the expense towards material and gamma-irradiation. Considering the low-cost, the developed formulation is suitable for large-scale commercialization.

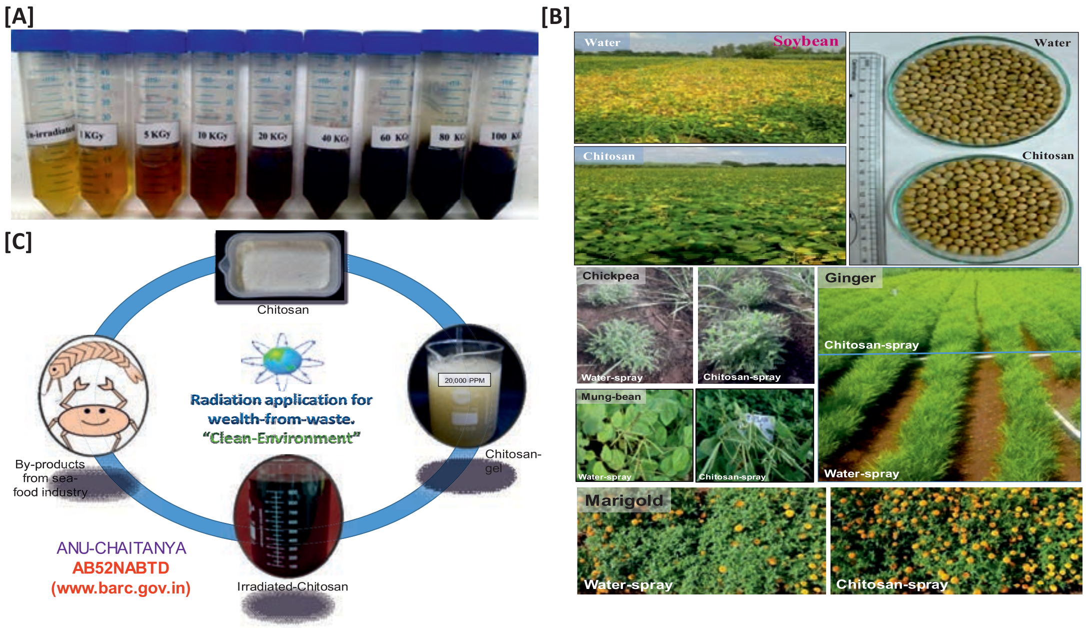

Fig. 4: Development of Anu-Chaitanya technology, using gamma irradiated-chitosan. [A] A range of radiation doses (1-100 kGy) were tested to depolymerize chitosan. [B] The foliar application of formulation developed using gamma irradiated-chitosan was found to have growth stimulatory effect in different crops including the horticultural plants. [C] A technology named “Anu-Chaitanya” has been developed, in collaboration with Vasantdada Sugarcane Institute, Pune, for converting chitosan into a versatile plant-growth bioregulator. The entire know-how of this technology is available for transfer through Technology Transfer & Collaboration Division, BARC.

Conclusion:

To sum-up, the present article describes the application of radiation-processed chitosan as an effective, economic and versatile bioregulator that can maximize the growth in both crops as well as horticultural plants. Further, the significant enhancement of crop yield has been obtained under the realistic field scenario, which is a key step to achieve sustainable agriculture. Since most of the chitosan is derived from the exoskeleton of crustacean animals, which is one of the major waste products of the seafood industry, the development of ANU-CHAITANYA technology can be seen as a typical example of both “wealth-from-waste” and “peaceful use of radiation”.

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