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ORIGINAL PAPER

Study on biological activity of chitosan after radiation processing

**Urszula Gryczka,
Helena Gawrońska,
Wojciech Migdał,
Stanisław W. Gawroński,
Andrzej G. Chmielewski**

Abstract. In recent years there is a trend in industry to limit the usage of chemical compounds. Natural polymers are new promising materials that possess important properties like biodegrability or lack of toxicity. Radiation processing of natural occurring polymers is an area of current research for development of new applications. The aim of this study was to determine the impact of ionizing radiation modification on bioactivity of a natural polysaccharide. Chitosan with different molecular weights was investigated as a biostimulator – a biologically active substance that stimulates some growth processes in plants. Chitosan 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. The effects of irradiation on the molecular weight of chitosan were investigated by viscosity and GPC measurements. Non-irradiated and irradiated chitosan at concentrations 0.001, 0.01, 0.1 and 1 g/dm^3 were used for greenhouse tests of its activity for growth promotion of *Salix viminalis* L. *var. gigantea* plant. Uniform rooted cuttings (20 per combination) were selected for the test and cultivated in aerated hydroponics culture containing Hoagland's nutrient solutions plus respective amounts of chitosan. After six weeks of plant exposure to chitosan, data of selected parameters of plant growth were collected. In most cases, except the highest concentration, both forms of chitosan 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 chitosan treated plants. The highest concentration of chitosan was stimulatory only for a number of roots and newly developed shoots while for other parameters was inhibitory. In comparable concentrations the stimulatory effect was greater for chitosan irradiated in comparison with the non-irradiated one.

Key words: chitosan • biostimulators • ionizing radiation • willow • biomass

U. Gryczka✉, W. Migdał
Experimental Plant for Food Irradiation,
Institute of Nuclear Chemistry and Technology,
1a Fasolowa Str., 02-482 Warsaw, Poland,
Tel.: +48 22 8638439, Fax: +48 22 8639935,
E-mail: sprupr@wp.pl

A. G. Chmielewski
Department of Nuclear Methods in Process Engineering,
Institute of Nuclear Chemistry and Technology,
16 Dorodna Str., 03-195 Warsaw, Poland

H. Gawrońska, S. W. Gawroński
Warsaw Uniwersity of Life Sciences,
159 Nowoursynowska Str., 02-787 Warsaw, Poland

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Introduction

There are several natural polymers such as carrageenan, alginate, chitin or chitosan that are classified as polysaccharides. One of the most promising is chitosan, deacetylated derivative of chitin, produced from shellfish wastes or fungi [15]. Irradiation of this polymer led the reduction of molecular weight by scission of glycosidic linkage. 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 [5]. Chitosan is used in agriculture, medicine and health care, as food additives and also in environment protection [8]. Investigation on radiation effect on polysaccharides like chitosan proved the increase of its bioactivity. Low molecular weight chitosan have been reported to possess novel features such as promotion of germination and stimulation of growth or protection of plant from fungal, bacterial and viral pathogens [9, 16, 17]. Biostimulators are a category of products which, by definition, increase plant productivity especially under unfavourable 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 [7].

Agriculture application of polysaccharides

The oligosaccharides derived from depolymerization of polysaccharides have been reported to exhibit growth-stimulating activity. 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 radiation degraded with a dose up to 500 kGy 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% [10]. Degraded polysaccharides such as alginate, chitosan or carrageenan, in concentrations from 20 to 100 μg/ml, increase tea, carrot or cabbage productivity by 15 to 40%. The use of degradeted polysaccharides can also reduce the damages caused by heavy metals and salts to plants [11]. Oligochitosan has been shown to be able to form in solution chelate complexes with metals such as vanadium that is well known to cause stress to plants. Chitosan irradiated with a dose in the range 70–150 kGy strongly affected the growth of wheat and rice plant and reduced damages caused by vanadium [16]. Low molecular weight chitosan was also reported as growth promoter for rape plants [4]. Chitosan oligomers (1 kDa) at a concentration of about 15 ppm have a positive effect on orchid plant growth. The effect increases with decreasing molecular weight [13]. Carbohydrates such as chitosan can also be used in plant protection from fungal, bacterial and viral pathogens both soil and foliar because they are strong antimicrobial agents [17]. Use of natural polymers to control plant pathogens may lead to the reduction in the use of fungicides. Ben-Shalom *et al*. [3] reported that spraying chitosan on cucumber plants inhibited the growth of gray mould caused by *B. cinerea*. Solution of chitosan in 0.1% concentration sprayed on tomatoes plants effectively protects them against viroid infection [14]. The antibacterial and antifungal effect of low molecular weight chitosan 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 [2]. In this study we attempt to modify chitosan by irradiation and check whether it enhances its biological properties as a plant growth promoter.

Experimental

Irradiation of chitosan

Chitosan powder from Shanghai Nicechem (95% deacetylation degree) was irradiated with an electron beam of energy 10 MeV from an Accelerator Elektronika 10-10 in the Institute of Nuclear Chemistry and Technology (INCT), Warsaw, to doses of 50–300 kGy [12]. Chitosan was irradiated in air. Effectiveness of degradation was measured by viscosity and GPC (gel permeation chromatography) methods. A Ubbelohde capillary viscometer was used for viscosity measurements. The value of reduced specific viscosity, defined as $(\eta/\eta_o - 1)\cdot c$, (η – viscosity of chitosan solution in concentration c [g/100 cm^3]; η_o – viscosity of solvent), was calculated.

Gel Permeation Chromatography system the Waters 1525 Binary HPLC Pump Waters, 2414 Refractive Index Detector and two columns WATERS Ultrahydrogel 500 and 250 were used.

Plant growth tests

The object in this study was willow (*Salix vinimalis* L. *var. gigantea*) grown in aerated hydroponics culture in a greenhouse using uniform wooden cuttings of 25 cm in length. Two cuttings were placed in a plastic container with 700 ml of Hoagland's nutrients solution [1] for two weeks for rooting. Uniformly rooted seedlings were then selected for study. They were grown for further six weeks in the same nutrient solution that was renewed after three weeks. Chitosan was applied as supplement during change of both nutrients in the following concentrations: 0.001, 0.01, 0.1 and 1.0 g/dm^3 (non-irradiated) and 0.01 and 0.1 g·dm^{-3} (irradiated). Controls were the plants grown in a Hoagland's nutrient solution without chitosan. At harvest data, the following parameters were collected: number of shoots and main roots, area of leaf blades (Leaf Area System, SKY UK), length of the longest shoots and total roots length (Root Length System, SKY UK). Also fresh and dry weights of newly developed shoots (with leaves) and of roots were recorded. The experiment was run in 20 biological replications (plant). Data, where appropriate, were subjected to statistical analysis ANOVA of Statgraphics Plus 4.1. Differences between the combinations were evaluated by LSD values of the Student t-test at $\alpha = 0.05$. Presented data are the mean ± SE, $n = 20$.

Results

Effect of irradiation on molecular weight of chitosan

The main effect of interaction of ionizing radiation with chitosan is polymer degradation. Radiation induced scission of 1–4 glycosidic bonds of chitosan chain [6]. It caused reduction in the molecular weight of the polymer. Figure 1 illustrates the relationship between viscosity of chitosan solution and irradiation dose. Rapid decrease of viscosity was recorded up to 100 kGy and then it slowed down and is related to molecular weight of polymer.

Figure 2 shows the gel permeation chromatography patterns of chitosan initial sample and after irradiation to doses 150 and 300 kGy. It can be observed that the main pick shifts towards longer retention time for higher irradiation doses, indicating the chain scission of the polymer.

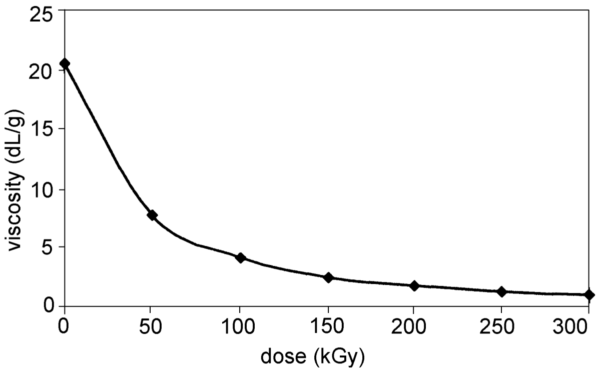

Fig. 1. Effect of irradiation on viscosity of chitosan.

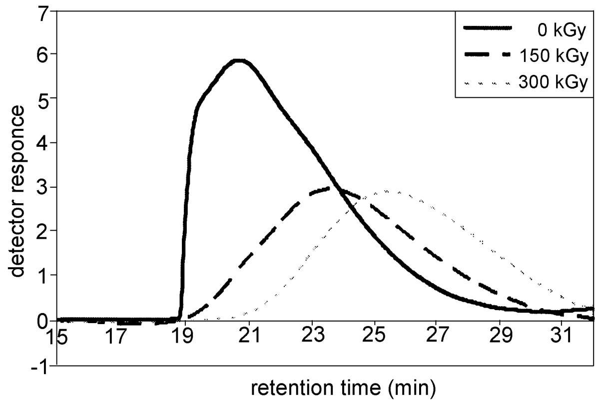

Fig. 2. The GPC chromatogram of chitosan samples.

Plant growth test results

Willow plants with a few exceptions were positively affected by treatments with chitosan and it was true both for root and growth above ground organs. Plants treated with chitosan developed greater system of roots. Number of well developed roots was greater at both concentrations of irradiated and at the highest of non--irradiated chitosan (Fig. 3). These roots were longer than in the control thus, in consequence, the total root length per plant was greater in chitosan treated plants (Fig. 4). Also the amount of biomass accumulated in roots was greater than in control plants (Fig. 5). An exception occurred for the highest chitosan concentration, at which both total roots length and biomass were lower than in the control plants (Figs. 4 and 5).

Similarly as in the case of roots, also the above ground organs were, with a few exceptions, also positively affected by chitosan. The number of newly developing shoots per plant was always higher than in the control plants (Fig. 6). The total plant leaf area increased due to chitosan treatment except for the concentrations 0.01 and 1.0 g·dm^{-3} (Fig. 7).

The amount of biomass accumulated in newly developed shoots together with leaves was, except for the highest concentration, greater in plants exposed to chitosan (Fig. 8). In consequence of the above described changes, the total plant biomass was, except for the highest concentration, also greater (Fig. 9) in chitosan treated plants than in the controls.

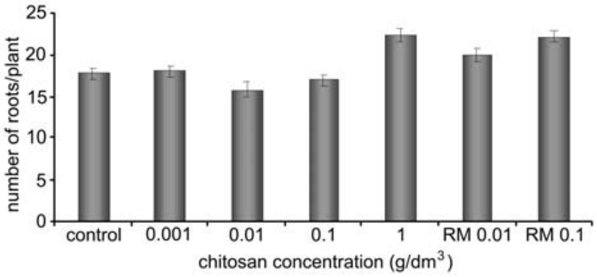

Fig. 3. Roots number in willow plants as affected by non--irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

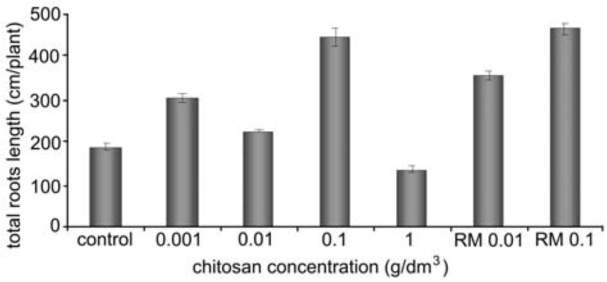

Fig. 4. Total roots length of willow plants as affected by non--irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

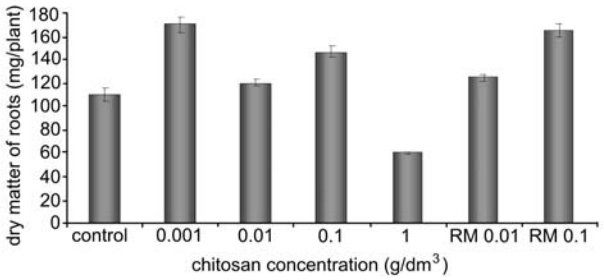

Fig. 5. Dry matter of roots of willow plants as affected by non-irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

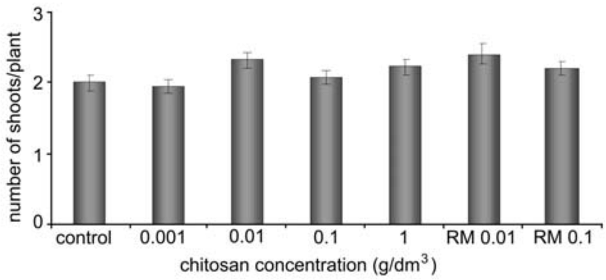

Fig. 6. Number of shoots in willow as affected by non-irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

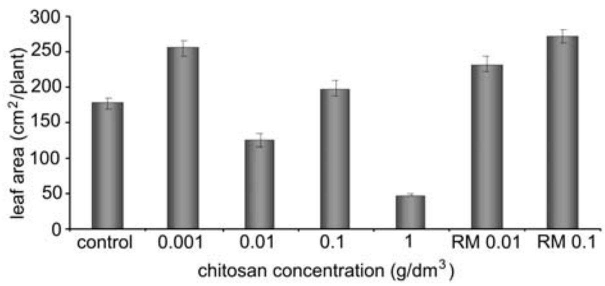

Fig. 7. Leaf area of willow plants as affected by non-irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

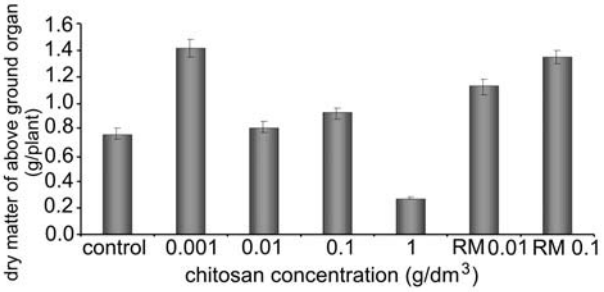

Fig. 8. Dry matter of above ground organs of willow plants as affected by non-irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

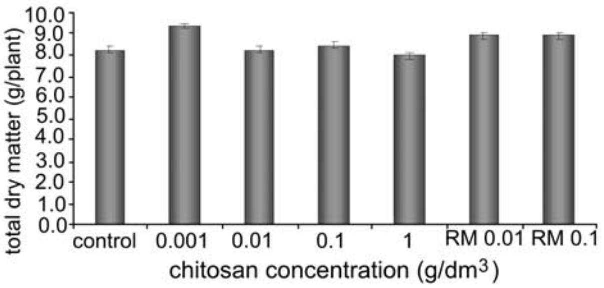

Fig. 9. Total dry matter of willow plants as affected by non-irradiated and irradiated (RM) form of chitosan. Data are the mean ± SE, $n = 20$.

In the case of irradiated chitosan the effect on all parameters was positive. When compare its effect to that exerted by non-irradiated form, in respective concentrations, the positive effect was, in all parameters, more evident. In most cases, except for the highest concentration, both forms of chitosan irradiated and non-irradiated had a stimulatory effect on leaf area, length of roots and newly developed shoots, fresh and dry weights leaf area, length of roots and newly developed shoots. The highest chitosan concentration 1 g/dm^3 was stimulatory only for a number of roots and newly developed shoots while, for the other parameters was inhibitory.

The results clearly demonstrated that chitosan effectively stimulates the developing of roots and shoots of plants. The growth stimulation effect was stronger for plants treated with irradiated chitosan than with unirradiated one.

Conclusions

It can be concluded that: (i) the viscosity and GPC analysis confirmed that irradiation is an effective method for chitosan degradation; (ii) chitosan exhibited the ability to stimulate plant growth; (iii) the most effective concentration of chitosan was 0.1 g/dm^3 in nutrient solution; (iv) both forms of chitosan had a stimulatory effect, but in comparable concentrations the stimulatory effect was greater for chitosan irradiated in comparison with the non-irradiated one.

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