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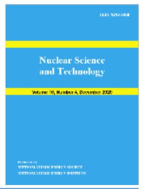

The growth promotion effect of microelement fertilizers containing low molecular weight chitosan and xanthan on radish (*Raphanus sativus* L.)

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Abstract: The effects of gamma radiation on viscosity and molecular weight (Mw) of chitosan and xanthan were studied to utilize them as components that can induce plant growth promotor (PGP) effect and prolong the contact of agrochemicals with crop in the foliar microelement fertilizers. Various fertilizers were prepared from two formulations of microelements, radiation degraded low molecular weight (Mw) chitosan (LCST) and xanthan (LXT) and their effects on the growth, yield and quality of radish grown on alluvial soil were measured. The experiment was arranged in randomized complete block design (RCBD) with 3 replications in experiment station. The results revealed that all development parameters of radish were much improved by foliar application of microelements and low Mw polysaccharides. The highest yield of radish root obtained with the plants treated with lower microelements and higher chitosan content (MF2). There are insignificant differences in total soluble solids, sugar and vitamin C content in the root harvested from the plants treated with the formulations supplementing the same amount of LCST, but the fertilizer composed of higher amount of microelements and chitosan (MF4) reduced nitrate residue in the root. It can be concluded that foliar microelement fertilizer containing low Mw polysaccharide can be applied to improve the growth, yield and quality of radish.

Keywords: *Gamma irradiation, xanthan, foliar microelement fertilizer, growth, radish.*

I. INTRODUCTION

Radish (*Raphanus sativus* L.) is a popular root vegetable grown all over the world. In Vietnam, radish is a winter crop with a short period of growth and development. The growth and yield of radish much depends on soil and nutrient condition. The leaves and tuberous roots can rapidly and uninterrupted grow with optimum fertilization. However, the overuse of chemical fertilizers will increase the production cost and contaminate the products with nitrate. Therefore, balanced fertilization of organic, inorganic and bio-fertilizers are required for the production of good quality radish [1]. Together with nitrogen (N), phosphorus (P) and potassium (K) are referred as the primary, and calcium (Ca), magnesium (Mg), and sulfur (S) are referred as the secondary macroelements, the plants requires microelements for their growth and defense [2,3]. Microelements not only stimulate physiological processes in plant, but also have a positive effect on its productivity. Therefore,

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application of microelement fertilizers increased the crop yield, especially for the plants grown on high pH because availability and uptake of micronutrients by plants decrease with increasing soil pH [4].

Polysaccharides are macromolecules with diverse structures from rod-shape (chitosan, xanthan), linear random coil (dextran, pupullan) to branched molecules (amylopectin in starch), though they are built up from very similar building blocks. This diversity in structure enable them to be versatile molecules with specific properties such as high viscous, good biocompatibility and biodegradability [5]. In addition, most polysaccharides are non-toxic and can easily be produced in large quantities at a suitable cost, so they have been intensively studied and utilized in agriculture [6]. However, polysaccharides can form defined structures such as helices, which are very low soluble in water influencing to their application. Fortunately, it was found that water solubility and some bio-activities of polysaccharides can be improved by decomposing into smaller molecules [7]. Several methods were developed to depolymerize or decompose the high Mw polysaccharides, and radiation degradation has been proved as potential way to prepare low Mw or oligo-saccharides [8]. Study on PGP effect of irradiated chitosan, Luan L.Q et al. indicated that the effect of low molecular weight (Mw) chitosan prepared by radiation degradation higher than that of initial one [9]. High elicitor activity of xanthan derived oligosaccharides was reported by Liu et al. [10]. Moreover, water solubility of low Mw xanthan was significantly improved by gamma irradiation [11]. Therefore, low Mw polysaccharides and oligo-saccharides have been widely used as plant growth promoters and/or elicitors in agriculture. In this field, xanthan can be applied as green adjuvant to control drift of fungicide, pesticide and fertilizer [12]. It also found that xanthan can acts as wetting agents for plant leaf. Thus, supplementation of low Mw chitosan and xanthan not only induce bioactivities, which promote the growth and reproduction, stimulate the immune system of target plants, but also ensure good spreadability of agrochemicals on the leaf surface, facilitation to their penetration into the plant cell wall.

In the present study, micronutrient fertilizers containing irradiated chitosan and xanthan have been prepared, and their effects on the growth and yield of radish grown on high pH soil were investigated.

II. MATERIALS AND METHODS

A. Materials

Chitosan from squid with a degree of deacetylation (DD) of ~ 75 % and Mν ~ 3×10^5 g/mol was bought from Chitosan Vietnam MTV Co., Vietnam. A commercial xanthan (Mν ~ 27×10^5 g/mol) at industrial grade was purchased from Deosen Biochemica, China. Fe-EDTA, Mn-EDTA and other chemicals were of industrial grade were purchased from Sinopharm Chemical Reagent and Guangdong Guanghua Sci-Tech Co., Ltd., China.

B. Radiation preparation of low Mw polysaccharide

Because the radiation degradation yield of polysaccharide in solution higher than that in powder state [5], the stock solutions of chitosan (3%) and xanthan (1%) were prepared in 2% acetic acid and distilled water, respectively, for preparation of radiation degraded low Mw polysaccharides. The solutions of chitosan and xanthan were irradiated with 25 and 55 kGy at the same dose rate of 4.5 kGy per hour under gamma Co-60 source at Hanoi Irradiation Center. After that, the irradiated solutions were precipitated in ethanol, washed and dried for preparation of low molecular weight chitosan (LCTS, Mv ~21,86 ± 3,25 kDa) and xanthan (LXT, Mv ~ 87,03 ± 9,74 kDa).

C. Microelement fertilizers containing low Mw polysaccharides and their effects on radish

Chitosan and xanthan are polyelectrolyte polysaccharides, which can form complex gels at certain conditions. However, the stable gel could not form at xanthan concentration below 0.5% [13]. Therefore, low Mw xanthan (15 ppm) and low Mw chitosan (50 and 75 ppm) were applied for preparation of microelement fertilizers as presented in Table I.

Table I. Compositions of foliar microelement fertilizers

Constituent Foliar fertilizer	Concentration (g.L-1)			
	MF1	MF2	MF3	MF4
Magnesium (Mg)	4	4	8	8
Boron (B)	0.8	0.8	14	14
Iron (Fe EDTA)	4	4	14	14
Manganese (Mn EDTA)	0.2	0.2	0	0
Copper (Cu)	0.32	0.32	0.64	0.64
Zinc (Zn)	0.32	0.32	0.6	0.6
Molybdenum (Mo)	0.32	0.32	1.8	1.8
Irrad. Chitosan (LCTS)	50	70	50	75
Irrad. Xanthan (LXT)	15	15	15	15
pH	6.22	6.11	5.84	5.92

The experiment was performed in the experimental station of the Research Center for Fertilizers and Plant Nutrients, Soil and Fertilizers Research Institute at Tien Yen, Hoai Duc, Hanoi. The experiment soil was non gley and alluvial soil having 0.13% available nitrogen, 65.0 and 70.1 ppm available phosphorous and potassium, respectively (Table II). The study was arranged in randomized complete block design (RCBD) with 3 replications (blocks) as Figure 1. Five plots include 4 treatments (sprayed with microelement fertilizers MF1-MF4) and control (sprayed with fresh water) were laid out within each block. Al least 3 seeds (Han-F1) were sown in the holes that regularly distributed in the plots on 20 February 2019. At three leaves stage, the seedlings were thinned and only three healthy seedlings were maintained during study. Radish were fertilized according to the local recommendation: manure 15 tons; 90 kg N (195 kg urea); 40 kg P_2O_5 (245 kg superphosphate) 90 kg K_2O (150 kg potassium chlorua) per ha. Each treatment was manually sprayed with one foliar fertilizer at first, 10-15 and 25-30 (vegetative stage) days after sowing.

Control	MF1	MF2	MF3	MF4
MF2	MF3	MF4	Control	MF1
MF1	MF2	MF3	MF4	Control

Fig. 1. Experimental layout for investigate the efficacy of foliar microelement fertilizer (MF) on the the growth and yield of radish plants

Table II. Characteristics of experimental soil (Tien Yen, Hoai Duc, Hanoi)

Parameter	Unit	Value
Moisture	%	22.5
Porosity	%	46.5
Density	g.cm^3	1.41
pH_{KCl}	-	7.1
Organic matter	%	1.69
Total nitrogen	%	0.13
Total phosphorus (P_2O_5)	%	0.14
Total potassium (K_2O)	%	1.54
Available phosphorus (P_2O_5)	ppm	65.0
Available potassium (K_2O)	ppm	70.1

Removal of weeds from the experiment area was manually done along with cultivation to manage pests and diseases on radish. The infestation level were estimated with mild, moderate and severe degrees as in Table IV. The roots were harvested on 15 April 2019. The yield and yield attributes were determined for each treatments. In which, yield attrbutes (average length of leaf, average length and weight of radish roots) were calculated from 10 plants, and total yield was determined by harvesting whole experimental plot. After removing small, infested and non-marketable roots, the marketable yield was determined. Typical roots were thoroughly washed, dried, and their quality parameters include dry matter (%), total soluble solids (°Brix), vitamin C (mg/100g) and nitrate residue were measured according to AOAC methods [14].

D. Statistical analysis

All data were statistically analyzed by analysis of variance (ANOVA), performed with IRRISTAT 5.1 software. The means were compared using the least significant differences test (L.S.D) at 0.05. [15].

III. RESULTS AND DISCUSSIONS

A. Radiation effects on viscosity and viscosity average molecular weight of radiation degraded polysaccharide

As one can see in Figure 2, the reduced viscosities of low Mw chitosan (A) and xanthan solutions much decreased by gamma radiation, and the reduction in the viscosity of xanthan was higher than that of chitosan. Differences in the reduced viscosities of chitosan and xanthan caused by differences in their molecular size and structure. In addition, difference in irradiation dose may also cause differences in the molecular properties of irradiated polysaccharides. From Figure 2, intrinsic viscosity [η] of radiation degrade chitosan and xanthan were calculated by extrapolating their reduced viscosities when the polymer concentration tend to zero. Because intrinsic viscosity of polymer solution and its molecular weight at constant temperature follows Mark-Houwink equation [16], these data suggested that molecular weight (Mv) of chitosan and xanthan were significantly decreased by gamma radiation. These reductions in viscosity and Mw of the irradiated polysaccharides can be explained by radiation degradation broke the glycosidic bonds in polymer molecules, formed the shorter chains with lower molecular weight and higher mobility in solution [17]. Thus, radiation degradation can be applied as a useful tool to prepare low molecular weight polysaccharides for further applications.

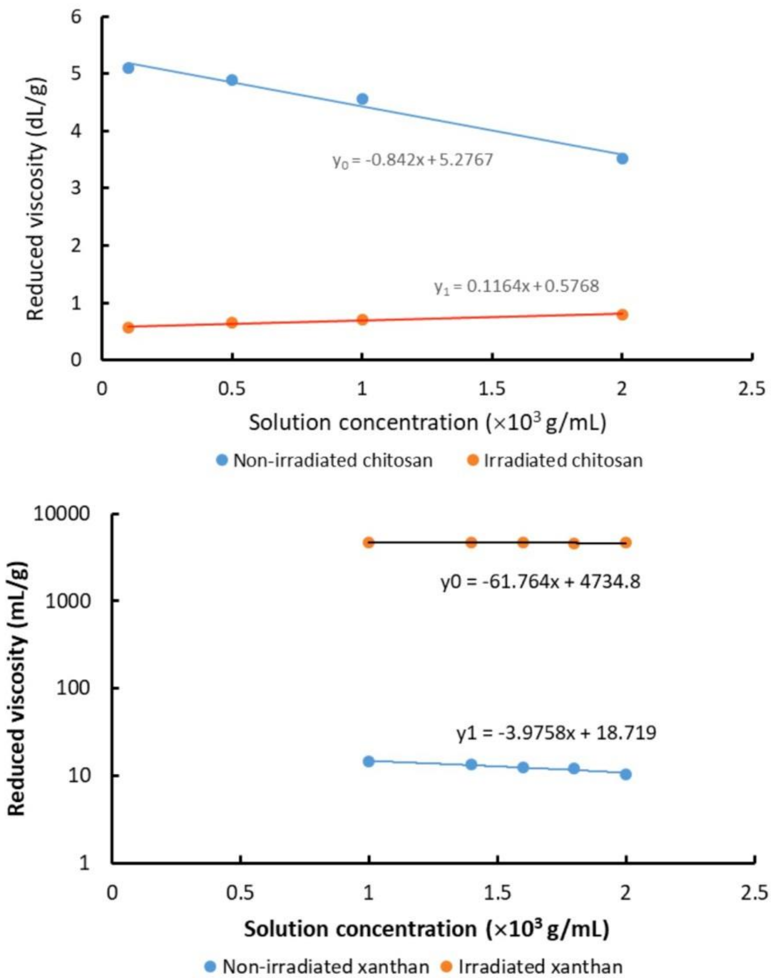

Fig. 2. Plots of reduced viscosity against concentration of chitosan, and xanthan

B. Effects of foliar fertilizers containing microelements and irradiated polysaccharide on radish growth

In this experiment, low Mw chitosan (LCST, Mv ~10-30 kDa) and xanthan (LXT, Mv ~ 60-100 kDa) have been prepared by radiation degradation for utilization as bioactive components in foliar microelement fertilizers, and the effects of the foliar fertilizer on the growth of radish were investigated. Table III showed all growth parameter and yield of radish root increased by foliar application of fertilizers containing microelements and low molecular weight polysaccharides. Not only the length of radish leaves, but also length, diameter and weight of radish root of the treated plants were significantly increased in compared with the control. The increment in the average root weight of the plants sprayed with foliar fertilizer containing low amount of microelements (MF1 & MF2) were higher than those of the others, though there are insignificant differences in the lengths of leaf and root among the treatments. It is obviously that the foliar fertilizers improved the growth and development of radish leaf and root. These may due to microelements and/or low Mw polysaccharides in the fertilizers enhanced nutrient uptake of plant, improved assimilation of organic matters and plant growth. As a consequence, both total and marketable yields of radish root increased by foliar application of microelement fertilizer.

Table III also indicated the yields of radish sprayed with lower microelements were higher, and the highest yield obtained with the radish treated with lower concentration of microelements and higher content of chitosan (MF2). Thus, supplementation of microelements and chitosan can improve plant growth and development, but higher concentration of microelements may not further affected. These results are consistent with other studies that chitosan promoted the radish growth [18-20].

Table III. Effects of foliar microelement fertilizers on yield and yield attributes of radish

Treatments	Ave length of leave (cm)	Ave length of root (cm)	Ave weight of radishroot (g)	Total yield (tons/ha)	Marketable yield (tons/ha)
Control	29.5^{b}	16.56^{b}	262.3^{b}	61.34^{b}	59.77^{b}
MF1	30.33^{a}	17.34^{a}	287.8^{a}	69.84^{a}	66.96^{a}
MF2	30.88^{a}	17.57^{a}	288.9^{a}	71.40^{a}	68.29^{a}
MF3	30.38^{a}	17.08^{ab}	269.6^{ab}	68.28^{a}	66.41^{a}
MF4	30.72^{a}	17.17^{a}	273.1^{a}	69.48^{a}	67.53^{a}
CV%	4.07	4.46	2.78	4.27	4.23
L.S.D (0.05)	-	13.58	28.74	7.44	5.51

Study on pest and disease infestation in radish during experiment period, the results in Table IV showed the infestation was controlled by foliar fertilizer. Degree of pest and disease infestation in all treated radish were lower than those of the control. As the results, the yield of radish treated with foliar fertilizer further increased. It was found that some microelements such as copper, zinc and boron could stimulate and regulate the immune system of the plant, so that promote the plant growth [21]. In addition, chitosan were reported to be active against viruses, bacteria and other pests in both monocotyledons and dicotyledons [22]. It was found that low molecular-weight chitosan (oligochitosan) is more effective in suppressing infection of the tobacco mosaic virus (TMV) in tobacco plants, and antiviral activity of chitosan increased as its molecular weight decreased [23]. Moreover, low Mw chitosan may induce the synthesis and accumulation of phytoalexin, a phyto-hormone that can produce adverse effects to the infesters and stimulating the generation of reactive oxygen species in plant [24]. Our results revealed that there are no scab rot can be observed with the radish root harvested from the plant sprayed with higher concentration of LCST (MF2 and MF4). Foliar fertilizer containing microelements and low Mw polysaccharides can protect radish from pests and diseases, therefore further improved the yield of radish root.

Table IV. Degree of pest and disease infestation in radish during experiment period

Treatment	Caterpillar	Painted bug	Mosaic root
Control	+++	++	++
MF1	++	+	+
MF2	+	+	-
MF3	+	+	+
MF4	+	+	-

Level: +++ Severe (≥ 40% leaf area infested); ++ Moderate (20-40% leaf area infested); + Mild (≤ 20% leaf area infested); – No infested

C. Effects of foliar fertilizers containing microelements and irradiated polysaccharide on the quality of radish root

The effects of various formulations of foliar fertilizers on some quality characteristics of radish root were measured and presented in Table V. The results indicated that the foliar application of microelement fertilizers affected on the quality of radish root. There are a slight increases in total soluble solids, total sugar and vitamin C content of the treated radish compared to the control. Foliar application of microelements and low Mw polysaccharide improved the nutrient uptake, then assimilation and accumulation of organic matters, sugar and vitamin C in radish root. Though the difference between two groups treated with different formulations of microelement was insignificant, the quality parameters of radish root seemed to increase with LCST content (MF2 and MF4). Thus, foliar application of microelements in combination with low Mw chitosan has further improved the accumulation of organic matter, sugar and vitamin C in radish root. Table V also revealed that foliar spraying of microelement fertilizer has significantly affected on the nitrate residues in radish root, and nitrate residue in the root may reduced by application of high amount of microelement and low Mw chitosan (MF4).

Table V. Effects of foliar microelement fertilizers on quality of radish

Treatments	TSS (°Brix)	Total sugar content (%)	Vitamin C (mg/100g)	Nitrate residue (ppm)
Control	3.98	1.14	28.32	478.64
MF1	4.32	1.18	27.92	464.16
MF2	4.53	1.21	30.74	480.82
MF3	4.16	1.16	29.31	462.89
MF4	4.63	1.18	30.6	432.10
CV%	8.32	13.35	7.53	8.27

IV. CONCLUSIONS

Radiation degradation can be applied to prepare low Mw polysaccharides to utilize as bioactive components in the foliar microelement fertilizers. The effects of foliar fertilizer were investigated with radish plants and the results showed that foliar fertilizer had significant effects on the growth, yield and quality of radish root. Foliar application of microelements and chitosan much increased the length of radish leaf and root, weight and yield of radish root, as well as accumulation of soluble solids, sugar and vitamin C in the root. Thus, the foliar application of microelement and low Mw polysaccharide plays an important role in the growth and development of radish, and therefore, the foliar fertilizer containing microelement and irradiated chitosan, xanthan can be applied to improve the plant growth and quality.

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TABLE AND FIGURE CAPTIONS

Table I. Compositions of foliar microelement fertilizers.

Table II. Characteristics of experimental soil (Tien Yen, Hoai Duc, Hanoi).

Table III. Effects of foliar microelement fertilizers on yield and yield attributes of radish.

Table IV. Degree of pest and disease infestation in radish during experiment period.

Table V. Effects of foliar microelement fertilizers on quality of radish.

Figure 1. Experimental layout for investigate the efficacy of foliar microelement fertilizer (MF) on the the growth and yield of radish plants.

Figure 2. Plots of reduced viscosity against concentration of (a) chitosan, and (b) xanthan.

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