Nucl. Sci. and Tech., Vol.12, No. 3 (2022), pp. 01-10

Nuclear Science and Technology

Journal homepage: http://jnst.vn/index.php/nst

Foliar Application of Micronutrients and Low Molecular Weight Polysaccharides on White Radish Grown in Impoverished Soil

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Abstract: Foliar application has been considered as the most effective way to supplement nutrients for crops. Besides macro and micro-nutrients, foliar fertilizers should contain surfactants to wet plant leaves, and other adjuvants to control spray and prolong the retention time of nutrients on the leaf surface. Having diverse structures and properties, some polysaccharides can not only be used as drift control or adhesion agents to improve performance on the leaf and reduce washout of agrochemicals by rainwater, but also be utilized as plant growth promoters (PGP) or immuno-stimulating agents in agricultural production. In this study, low molecular weight chitosan and xanthan obtained by radiation degradation have been added to the microelement fertilizers to control diseases, promote the growth and development of vegetables, and further increase their nutrient uptake. Field trials on white radishes grown on impoverished soil revealed that the resulting foliar fertilizers significantly reduced the infection of popular pests and diseases, remarkably promoted the growth and development of the radish plants. The yield attributes and marketable yield of radish from all treatments were much higher than those of the control. The highest yields were 61.99 and 64.94 tons per ha in season 2019 and 2020, respectively, in the plants treated with the fertilizer containing both chitosan and xanthan. These results suggest that the fertilizer composed of micronutrients and low molecular weight chitosan and xanthan could be applied for better vegetable crops.

Keywords: *Foliar fertilizer, microelements, chitosan, xanthan, yield, quality*.

I. INTRODUCTION

Nutrition is one of the most important factors that govern the growth, yield, and quality of crops. The demand for nutrients of the plant largely depends on the climatic conditions, fertility and nature of soil, and its variety. Short-duration and quick-growing crops such as vegetables continuously require additional nutrients through fertilization for optimal growth and yield. Although chemical fertilizers have been considered as an effective means to provide essential nutrients for plants for a long time, the overuse of inorganic fertilizers in agricultural production may contaminate the food products and cause severe problems for the environment and human health. As the plants can only partly absorb them, more than 50% of chemical fertilizers enter to the environment through run-off, leaching into the soil or even volatilizing into the air [1]. For example, the plants can only absorb up to 50% of the N fertilizer applied in ideal conditions, 2-20% gets volatilized, 15-25% reacts with organic compounds in the soil colloids and the

https://doi.org/10.53747/nst.v12i3.333
Received 22 March 2022, accepted 10 January 2023

remaining 2-10% interferes with surface and groundwater [2]. Therefore, a balanced fertilization composed of inorganic, organic, or biofertilizers must be developed to reduce the use of chemical fertilizers [3].

In addition, influences of the fertilizer strongly depend on its application, and foliar fertilization was proved as an effective approach to supplement agrochemicals for crops because it requires less resources, independent of soil conditions, and can be quickly and efficiently absorbed by plants. Foliar application is a method to immediately deliver nutrients, especially micronutrients to the plant tissues and organs. Foliar application of macro and micro-nutrients during crop growth can further improve the yield and quality of crops [4].

Although foliar fertilization does not completely replace soil fertilization, it is found that foliar application of micronutrients is the best way to immediately replenish their deficiencies [5]. Because the micronutrients are not fixed or diluted in large volumes of soil, they can quickly penetrate into the leaf and directly enter into metabolic processes within the plants for optimal growth [6].

Since agrochemicals may not be completely absorbed by plants and discharged into the environment, especially in heavy rain, some adjuvants should be added to the liquid fertilizers to control the spray drift, prolong the retention period of the nutrients on the leaves for their uptake. Polysaccharides can not only be applied as thickening agents, which modify the viscosity of spray liquid, but some natural polysaccharides have also been utilized as drift control agents due to their high viscosity [7]. In addition, polysaccharides and their derivatives, especially oligosaccharides can be utilized as plant growth promoters, regulators and elicitors to promote plant growth and development as reported elsewwhere [8-11]. For example, chitosan, the most important derivative of chitin, has been intensively studied and widely applied to induce growth promotion effects in tomatoes [9], daikon radishes [10], cabbage [11], soybean sprouts [12]. It is also reported that chitosan can activate the plant cells, and improve their resistance to harmful insects and diseases [13].

However, the poor water solubility of polysaccharides and their highly viscous aqueous solutions somewhat limit their application in practice. Some methods have been developed to break down large molecular chains into smaller fractions to improve their application. Recently, radiation degradation was recognized as a potential way to decompose polysaccharides to low molecular weight fractions or oligosaccharides, which can be applied as plant growth promoters. Kume et al. reported that the radiation-degraded polysaccharides can induce the production of phytoalexins – antimicrobial and antioxidative substances that promote plant disease resistance [14]. It was also found that, water solubility of xanthan was significantly improved by gamma radiation degradation [15], and radiation-degraded xanthan can be used as an adhesive to improve the efficiency of foliar fertilizer [16]. Thus, radiation-degraded polysaccharides can be utilized as the main bioactive components in foliar fertilizers.

Radishes (*Raphanus sativus* L.) are one of the fastest-growing vegetables, taking just four to six weeks to reach harvest depending on their varieties. The appropriate application of fertilizers is essential for ensuring a high yield and good quality of radish roots [17]. In Vietnam, radish is a commonly winter vegetable with very high nutritional demands and relatively high production potential [18]. The growth and yield of radish much depends on soil and nutrient conditions, and the plants require both macro and micro-nutrients for optimal growth and yield. In the present study, foliar fertilizers containing the same formulation of micronutrients and various radiation-degraded polysaccharides have been prepared for radishes grown in impoverished soil, and their effects on the growth and development of the radishes were investigated.

II. CONTENTS

Materials: High molecular weight chitosan (Average viscosity molecular weight Mν ~ 250 kDa, deacetylation degree DDA ~ 85 %), and xanthan (Mν ~ 3000 kDa) were purchased from Chitosan Vietnam Co., (Kien Giang, Vietnam) and Deosen Biochemical Ltd., (Shandong, China). Micronutrient salts were bought from Duc Giang Chemical Group (Hanoi, Vietnam). A Korean (F1) radish variety was kindly supported by the Research Center for Fertilizers and Plant Nutrients, Soil and Fertilizer Research Institute (SFRI), and planted in the experimental station at Trang Viert, Me Linh, Hanoi. NPK and organic manure are locally popular fertilizers.

Obviously, the impoverished soil in this study is acidic and relatively poor in organic matters and nutrients, so it need to be supplemented with micronutrients together with NPK and manure fertilizers for optimal growth and yield of vegetable crops in general and radish in particular. Therefore, a formulation of micronutrients composed of Ca, Mg, B, Fe, Mn, Cu, Zn and Mo has been established based on the nutrient requirements of vegetable crops as indicated in Table I.

Table I. Characteristics of experimental soil
(initial soil samples were collected from 0 -15 cm depth from surface)

Parameter	Unit	Value
Moisture	%	16.8
Porosity	%	52.6
Density	$g.cm^3$	1.14
pH_{KCl}	-	4.8
Organic matter	%	1.18
Total nitrogen	%	0.19
Total phosphorus (P_2O_5)	%	0.04
Total potassium (K_2O)	%	0.04
Available phosphorus (P_2O_5)	ppm	53.5
Available potassium (K_2O)	ppm	53.1

Preparation of micronutrient fertilizers for foliar application: Low Mw chitosan (Mν ~ 10-30 kDa, DA ~ 85%) has been prepared by radiation degradation as described in our previous study [19]. Xanthan was dissolved in distilled water overnight into a 1.5% solution, then irradiated at 50 kGy to obtain the xanthan solution having Mν within 60-100 kDa. Four microelement foliar fertilizers were prepared with or without radiation degraded chitosan and xanthan as presented in Table II. The amounts of microelement salts, chitosan and xanthan have been predetermined for raddish as indicated in our previous study [20]. Various amounts of these radiation-degraded polysaccharides were added to the aqueous solution of micronutrients, then homogenously mixed into foliar fertilizers.

Experimental design and treatment: The experiment was set up with a randomized complete block design (RCBD) with five treatments and three replications. The experiments were performed according to the recommendation of the Ministry of Agriculture and Rural Development and 108/2017/ND-CP Decree, at the SFRI experimental station (Trang Viet, Me Linh, Hanoi, Vietnam) during the 2019 and 2020 growing seasons. Soil samples were collected and analyzed by the Division of Land Use Research, SFRI. The control plants (CON) were fertilized with decomposed manure (5 tons), and NPK (70 kg N; 30 kg P_2O_5; 80 kg K_2O) for one hectare. All foliar fertilizers were applied for the various treatments at the same rate of 5 L per 1 hectare.

Table II. Compositions of foliar microelement fertilizers

Constituents ($g.L^{-1}$)	Foliar Microelement Fertilizers			
	MIE	**MCT**	**MXT**	**MCX**
Ca ($CaCl_2$, %)	0.3	0.3	0.3	0.3
Mg ($MgSO_4.7H_2O$, %)	0.2	0.2	0.2	0.2
Fe ($FeSO_4.7H_2O$)	2500	2500	2500	2500
Mn ($MnSO_4.5H_2O$)	1000	1000	1000	1000
Cu ($CuSO_4.5H_2O$)	1500	1500	1500	1500
Zn ($ZnSO_4.2H_2O$)	1600	1600	1600	1600
Mo ($Na_2MoO_4.2H_2O$)	450	450	450	450
B ($Na_2B_4O_7.10H_2O$)	3500	3500	3500	3500
Chitosan* (%)	0	1,25	0	1,25
Xanthan* (%)	0	0	0,3	0,3
pHH_2O	6.2	6.0	6.3	6.2

* Radiation-degarded low molecular weight polysaccharide; MIE = Micronutrient Fertilizer; MCT = Micronutrient Fertilizer containing chitosan; MXT =: Micronutrient Fertilizer containing xanthan; MCX = Micronutrient Fertilizer containing both chitosan and xanthan.

The land was well ploughed and harrowed, leveled with raised beds of 15-20 cm in height. Before sowing, the furrows were made to fertilize. Radish seeds were sown at 2-3 seeds per hole with 15×25 cm inner and intra row spacing on the prepared beds of 1.5×8 m (12 m^2). Plants were thinned to 1 at the 3-leaf stage, soil was turned up to cover radish root. Irrigation was applied as per crop requirements.

Measurements: Infestation levels of pests and diseases were also observed and their severities were recorded based on the AVRDC (World Vegetable Center) guidance to estimate the impacts of foliar fertilizers on the resistance of radishes. Before harvesting, the plant height, average number of leaves, and length of the largest leaf were recorded. During harvesting, the length, diameter and weight of radish roots were measured and the yield were determined for each treatments.

After that, five roots from each treatment were randomly selected for sampling, and their quality parameters including dry matter (%), total soluble solids (°Brix) and vitamin C (mg/100g) were measured according to AOAC methods.

Statistical analysis: All data were statistically analyzed by analysis of variance (ANOVA) using EXCEL and IRRISTAT 5.0 software. The least significant differences test (L.S.D) was applied for the mean separation (Kwanchai and Arturo, 1984).

RESULTS

Effects of foliar fertilizers on the radishes grown in impoverished soil

Growth and development of radishes were recorded with all treatments during the first season. Results in Table III showed the major growing parameters of all treated radishes were higher than those of control, suggesting that foliar supplementation of micronutrients promoted the growth and development of vegetables. Furthermore, plant height, number of plant leaves and length of the largest leaf of radishes were increased by foliar application of radiation degraded polysaccharides, especially in the plants treated with chitosan. The foliar fertilizers improved the growth and development of radishes. The maximum plant height, number and length of the largest leaves, were 29,72 cm, 14,43 and 29,61 cm, respectively, obtained with the radishes treated by MCX, the foliar fertilizer containing micronutrients, and both radiation degraded chitosan and xanthan.

Table III. Effects of foliar microelement fertilizers on the growth and development of radishes

Treatments	Plant height (cm)	Number of plant leaves	Length of the largest leave (cm)
CON	22.03^{c}	12.66^{c}	26.99^{b}
MIE	23.73^{bc}	13.52^{b}	27.26^{ab}
MCT	27.13^{ab}	13.96^{a}	28.59^{a}
MXT	25.02^{b}	13.76^{a}	28.09^{a}
MCX	29.72^{a}	14.43^{a}	29.61^{a}
CV*(%)	2.83	4.93	2.02
L.S.D (0.05)	1.31	1.23	1.03

* CV: = Coefficient of variation; LSD(0.05): = Least significant difference at 0.05. Different letter superscripts in the same row indicate a significant difference ($p < 0.05$).

Table IV. Pest and disease infestation on radish during experiment period

Treatments	Caterpillar	Radish flea beetle	Mosaic root rot
CON	+++	+++	++
MIE	+++	++	+
MCT	+	++	-
MXT	++	++	+
MCX	+	+	-

Infested level: +++ Severe; ++ Moderate; + Mild; – Not sure.

In this experiment, the main insect pests in the radish plants were caterpillar and radish flea beetle. Generally, these pests and diseases are controlled by synthetic insecticides, but the use of chemicals may induce negative impacts to the quality of radish roots. Without insecticide, the radishes were not only damaged by insect pests, but also infested by fungal diseases as observed in Table IV. Depending on the foliar application, the damage levels were different, and the lowest damages were recorded with the plants sprayed with the formulation containing both chitosan and xanthan.

Table V. Effects of foliar microelement fertilizers on yield attributes of radish

Treatments	Root length (cm)	Root diameter (cm)	Root weight (g)
CON	20.47^{b}	5.93^{ab}	478.7^{c}
MIE	20.85^{b}	6.02^{ab}	489.0^{bc}
MCT	21.32^{a}	6.30^{a}	529.0^{a}
MXT	21.08^{a}	6.28^{a}	517.7^{b}
MCX	21.98^{a}	6.53^{a}	546.3^{a}
CV*(%)	2.13	8.12	8.44
L.S.D (0.05)	0.82	0.92	78.66

* CV: = Coefficient of variation; LSD(0.05): = Least significant difference at 0.05. Different letter superscripts in the same row indicate a significant difference ($p < 0.05$).

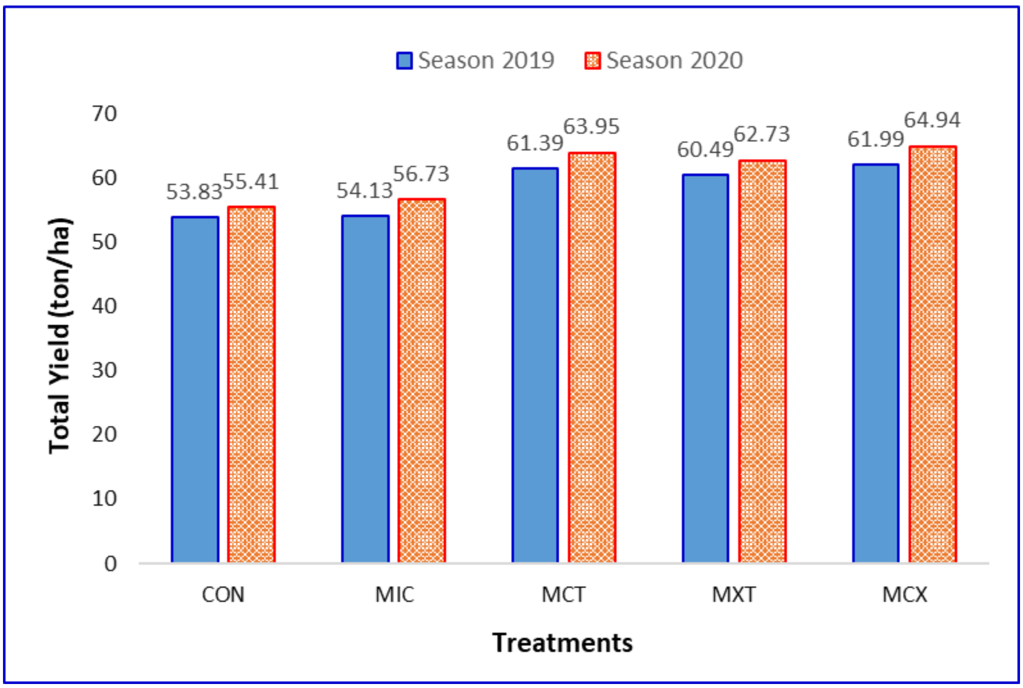

Fig. 1. Total yields of radishes supplemented with various foliar fertilizers

The yield attributes and yield of radish root were determined with all treatments and the results were showed in Table V and Figure 1, respectively. As it can be observed from Table 5, the yield attributes of radishes supplemented with foliar fertilizers were significantly increased. Among them, yield attributes of the radishes treated with chitosan were higher than others. Although differences in yield attributes between the radishes treated with the fertilizers containing low Mw chitosan (MCT and MCX) were not statistically significant. Maximum root length and diameter were recorded in the radishes treated by MCX. Likewise, maximum weight of roots was observed with these radish plants although the increment in root weight was rather higher than that in root sizes. As results, the highest root yields obtained with the radishes treated by MCX, a foliar fertilizer containing both low Mw chitosan and xanthan, were 61,99 and 64,94 $t.ha^{-1}$ in 2019 and 2020, respectively (Figure 1).

Table VI. Effects of foliar microelement fertilizers on quality of radish root

Treatments	TSS (°Brix)	Reduced Sugar content (%)	Vitamine C content (mg/100g)
CON	3.92^{c}	1.43^{c}	27.68^{c}
MIE	4.22^{b}	1.59^{b}	28.81^{b}
MCT	4.49^{a}	1.70^{a}	29.75^{b}
MXT	4.37^{b}	1.69^{a}	30.16^{a}
MCT	4.55^{a}	1.77^{a}	30.96^{a}

Values represents mean of three replecations. Different letters in columns indicate significant differences between treatments according to LSD test ($P < 0.05$).

Application of foliar fertilizers composed of micronutrients and radiation degraded polysaccharides also affected the quality parameters of radish root as indicated in Table VI. There were significant increases in total soluble solids (TSS), reduced sugar and vitamin C content in the treated radishes compared to the control, suggesting that foliar fertilizers improved the nutrient uptake of radish, and further promoted the assimilation and accumulation of organic matters, sugar and vitamin C in the radish root. However, these quality parameters measured with the radishes treated by the fertilizers containing different amounts of low Mw polysaccharides were not significantly different. Thus, supplementation of polysaccharides does not seem to change the quality of radish root in practice.

DISCUSSION

The growth parameters of the radishes sprayed with foliar fertilizers composed of micronutrients and low Mw polysaccharide significantly increased compared with those of the control. These results are consistent with other studies that found chitosan promoted the growth of radishes [10, 21, 22]. Low Mw chitosan acted as a plant growth promoter (PGP) for radishes, whereas xanthan acted as an additive that controlled spray drift and prolonged the retention period of micronutrients on the leaf surface [16]. However, though the highest radish yield was recorded with the plants treated by fertilizer containing both chitosan and xanthan (MCX), the addition of xanthan seemed not to further improve the PGP effect of chitosan due to the differences in yield and yield components between the plants treated by MCT and MCX were insignificant.

Depending on soil and cultivar, pathogen diseases from leaf spots to wilting, cankers, and wet rot may be observed on vegetables. Radishes can be also be attacked by insect pests. Obviously, the damage levels were significantly reduced by the foliar application of micronutrient fertilizers. Even mosaic root rot, a typical bacteria disease, was no longer observed in the radishes treated with chitosan (MCT and MCX fertilizers). Chitosan inhibited the growth and spread of pathogen on vegetable by stimulating their immune responses [23]. It may be because low Mw chitosan induced the synthesis and accumulation of phytoalexins, which can cause toxic effects on the infesters and stimulate the generation of reactive oxygen species in plants [24]. Therefore, the obtained foliar fertilizers of micronutrients, especially those containing low molecular weight chitosan, can be applied to protect radishes from insect pests and diseases, thus improving the growth and yield of radishes grown in impoverished soil.

In both the 2019 and 2020 growing seasons, the yield of radish root was much increased by supplementing with foliar fertilizers. It is because the plants were not only provided with essential nutrients and bioactive substances for their growth, but some microelements in the foliar fertilizer such as copper, zinc and boron could also stimulate and regulate the immune system of plants, so that further increasing their yield [25]. Chitosan not only promote plant growth, but can also act as a biological signal in plant cells to stimulate and regulate their defense systems, thus further improving the crop yield [26]. In addition, high viscosity and adhesion of xanthan increase the nutrient uptake through plant leaves and further improve the efficiency of foliar fertilizers for radish. Figure 1 also revealed that the root yields of all treatments recorded in the second season were higher than those in the first season. This may be due to the differences in climate conditions.

III. CONCLUSIONS

New foliar fertilizers have been prepared from suitable formulations of micronutrients, and radiation degraded chitosan and xanthan for the radishes grown in impoverished soil. The results revealed that obtaining foliar fertilizers largely promoted the growth and development of radish plants. Foliar micronutrient fertilizers, especially those containing low Mw chitosan, inhibited the infestation and spread of common pests and diseases in radish leaves and root. All yield attributes and root yield of treated radishes were significantly higher than those of the control. The highest root yield was recorded in the radishes sprayed with the micronutrient fertilizer containing both low Mw chitosan and xanthan. There were no significant differences in the quality of radish roots between the treated plants, though their quality parameters were significantly improved by foliar supplementation of micronutrient fertilizers. Thus, foliar fertilizers composed of micronutrients and low Mw polysaccharides can be applied to the radishes grown in impoverished soil to promote their growth and improve the yield and quality of radish root.

ACKNOWLEGEMENT

This work was kindly supported by the Vietnam Ministry of Science and Technology under government project of DTDLCN.19/16. The authors thank to Hanoi Irradiation Center, and the Soils and Fertilizers Research Institute for their supports.

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