THE EFFECTS OF APPLYING CHITOSAN OF DIFFERENT MOLECULAR WEIGHTS ON THE GROWTH AND QUALITY OF KAMCHATKA BERRIES (*LONICERA CAERULEA L.*): PART 1

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Abstract
Kamchatka berries contain many valuable organic and mineral compounds. However, the ripe berries are not very firm and have low transport resistance. We aimed to study the effect of chitosan solutions of different molecular weights on the growth, yield, and quality of Kamchatka berries. Chitosan with lower molecular weights, 3 and 12 kDa, stimulated plant growth, while those with molecular weights above 50 kDa reduced plant growth compared with control shrubs. Chitosan 125 and 500 kDa increased Kamchatka berry yield. In addition, chitosan 125 kDa increased fruit weight throughout the yield period. Fruit sprayed with chitosan 3, 5, and 50 kDa were dark in colour at harvest. Chitosan 3, 5, 12, and 50 kDa increased fruit/skin resistance to mechanical damage. Finally, chitosan 3, 5, 50, and 950 kDa increased average fruit firmness. Overall, we recommend higher molecular weight chitosan to increase yield and lower molecular weight chitosan to increase firmness and resistance to damage.

Keywords: *shrub growth, yield, fruit firmness, fruit size*

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1. Introduction

Kamchatka berries or blue honeysuckle berries (*Lonicera caerulea* L.) have been widely planted for many years in Russia, Japan, and Canada [1]. So far, these berries are only planted on a small scale in Poland, but interest in this plant is gradually increasing. Its full frost hardiness (down to –40°C) and relatively low climatic requirements mean that the species can be planted successfully throughout Poland [2], especially as new varieties from various breeding centres are available [3]. The first harvest can be obtained several days before the strawberry and raspberry harvests, which means that these are the first fruit from indigenous production to appear on the market. The undoubted disadvantages are the dropping of ripe fruit and their relatively low firmness and therefore low transport resistance [4]. The retention of firmness after harvest is one of the most important indicators of freshness and consumer attractiveness. Many factors reduce the shelf life of fruit [5].

Over the last few years, numerous preparations that stimulate plant growth and development have appeared on the Polish market. These products could address consumer demands and improve the quality characteristics of plants and fruit [6]. However, the use of plant protection products and synthetic fertilisers is one of the main causes of environmental degradation. In recent years, measures have been taken in the countries of the European Union to minimise or prevent the effects of excessive and often irrational chemigation of agricultural production [7]. Chemical plant protection, when possible, should be replaced by biological, physical, and agrotechnical methods [8]. To this end, there is a continuous search for the best conditions to ensure plant growth and development without causing biotic and abiotic stresses [9].

One substance of natural origin used in agricultural production is chitosan (CH). This polysaccharide of repeating β(1→4)–d–glucosamine and *N*–acetyl–d–glucosamine units is the second most abundant polysaccharide in nature after cellulose [10]. It is derived from crustacean shells and other sources such as insects, fish scales, and fungi [11]. It is a non-toxic and biodegradable compound of natural origin that is obtained by deacetylation of chitin [12]. This compound is characterised by unique properties such as bioactivity and biocompatibility [13, 14]. When applied to plants, CH can increase yield, reduce transpiration, and induce several metabolic changes that result in plants becoming more resistant to viral, bacterial, and fungal infections [15–17]. CH stimulates vital processes of plants on every level of biological organisation: from single cells to tissues, through physiological and biochemical processes all the way to changes at the molecular level related to gene expression [18, 19]

CH covers a wide group of polycationic macromolecular compounds differing mainly in their molecular weight and degree of polydispersity. These parameters influence most of the biological properties and functions of CH [20]. CH has low solubility in water and is therefore subjected to chemical modifications. Derivatives are formed that are allow it to dissolve more easily [21] – for example, water-soluble oligomers of CH lactate. They are characterised by short chains and a low molecular weight [22]. The efficacy of CH-based formulations varies considerably, and they need to be tested for use in planting.

We investigated the effect of foliar-applied CH on bush growth, yield, and fruit quality of Kamchatka berries.

2. Materials and Methods

2.1. Characteristics of the Research Area and Plant Material

The experiment was conducted at the Horticulture Department of the West Pomeranian University of Technology in Szczecin. Blue honeysuckle bushes, cultivar Zielona, were planted at a spacing of 3 × 1 m in clay soil, classified as Class III, with pH 6.2–6.5. The soil had a high abundance of potassium and phosphorus and a medium abundance of magnesium, so only nitrogen fertilisers at a dose of 45 kg nitrogen were applied. The plants were irrigated with a drip line according to the soil tensiometers.

Every year, before the start of vegetation, the bushes were pruned and damaged. The dry and oldest shoots were removed. Before the berries were fully coloured, a net was laid over the plant to protect it from birds.

2.2. Production of CH and Film Preparation

Chitin was obtained by demineralising, deproteinising, and deodourising shrimp (*Farfantepenaeus brasiliensis*) waste.

The plants were treated with CH with molecular weights of 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. The degree of deacetylation was 85%. The plants were sprayed once after the start of vegetation and three times a week after the end of flowering. The same plants were sprayed annually with a 0.2% solution of CH until the leaves were fully wetted. Control plants were sprayed with distilled water only. No other chemical plant protection was applied during the experiment. The berries were harvested several times (5–7 times) in June as they ripened.

2.3. General Fruit Parameters

Every year, the fruit yield and weight were measured (RADWAG WPX 4500 ± 0.01 g, Poland). The CIE *L*a*b** parameters was measured using a KonicaMinolta CM-700d spectrophotometer (Chiyoda, Japan). The colour parameters and indices were averaged over 50 measurements. The firmness and puncture resistance of the skin were measured with a FirmTech2 apparatus (BioWorks, USA) on 100 randomly selected berries from three replicates; the values are expressed as a gram-force causing the fruit surface to bend 1 mm [23].

2.4. Statistical Analysis

All statistical analyses were performed with Statistica 12.5 (StatSoft Polska, Poland). The data were subjected to one-way variance analysis (ANOVA). The Tukey least significant difference (LSD) test was used to compare the means between the groups. Significance was set at $p < 0.05$.

3. Results and Discussion

We found a significant but inconclusive effect of the applied CH solutions on bush growth, yield, and Kamchatka berry quality (Table 1).

Regarding plant growth during the vegetation period, CH with a higher molecular weight, from 50k, reduced plant growth compared with control shrubs. In contrast, CH 3k and 12k stimulated plant growth: the plants were taller and wider. CH 12k and 21k led to larger leaves and the longest annual shoots, indicating the stimulating nature of these agents. The leaves were also greener, especially those sprayed with CH 125k, compared with the control plants (Figure 1).

Leaf colour is highly correlated to the chlorophyll content [24]. Dzung *et al.* [25] reported an increase in the chlorophyll content in leaves after CH application. This may be due to increased plant uptake of nutrients [19]. The effect of the CH molecular weight may depend on the plant species. Luan *et al.* [26] showed that low-molecular-weight CH

(16k) had the strongest growth promotion effect on plants in vitro, while Lee *et al.* [27] reported that CH > 1000k had the most beneficial effect on soybean seedlings.

Low-molecular-weight CH – especially 12k, which stimulated bush growth the most, – did not increase fruit yield compared with the control (Table 2). Shrubs sprayed with CH 21k had the highest yield, but the berries was small. We also found a significant increase in the yield for plants sprayed with CH 125k and 500k. However, these CH formulations did not stimulate vegetative growth in the plants (Table 1). CH 125k also contributed to an increase in fruit weight over the entire yield period. The producer is looking for ways to increase fruit volume, but the consumer expects large fruit, which cannot always be achieved.

Table 1. The growth of Kamchatka berry bushes treated with different molecular weights of chitosan.

Chitosan	Increase during the growing season [cm]			Leaf area [cm^2]
	Height	Width	Annual shoots	
Control	23.8^{c}	24.3^{c}	17.8^{bc}	15.1^{ab}
3k	33.6^{d}	29.7^{cd}	24.3^{c}	14.1^{ab}
5k	22.4^{c}	21.6^{bc}	22.2^{c}	16.0^{bc}
12k	36.4^{d}	39.1^{d}	27.5^{d}	18.6^{d}
21k	18.2^{bc}	20.2^{bc}	25.9^{cd}	17.6^{cd}
50k	9.8^{ab}	9.1^{a}	17.3^{bc}	14.8^{ab}
125k	11.2^{ab}	12.1^{ab}	16.2^{b}	15.4^{b}
500k	14.0^{ab}	8.1^{a}	7.3^{a}	15.2^{ab}
950k	5.6^{a}	9.4^{a}	9.7^{a}	13.3^{a}

Note. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. Mean values with the same letter do not differ significantly ($p > 0.05$) according to the Tukey test.

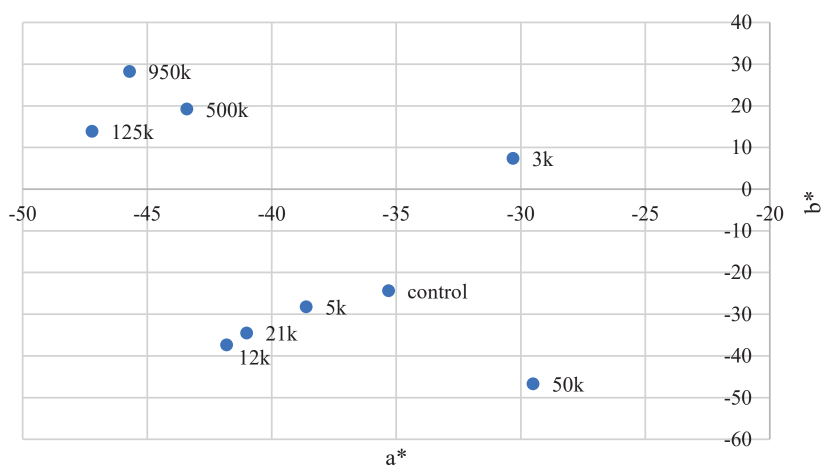

Figure 1. The colour of Kamchatka berry bush leaves treated with different molecular weights of chitosan. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa.

Table 2. The fruit yield and weight of Kamchatka berry bushes treated with different molecular weights of chitosan.

Chitosan	Yield [kg/shrub]	Weight of one fruit per harvest date [g]			
		Mean	Beginning of harvest	Middle of harvest	End of harvest
Control	2.27[ab]	1.46[ab]	1.17[a]	1.91[bc]	1.31[abc]
3k	2.45[bc]	1.47[ab]	1.23[a]	1.65[a]	1.53[de]
5k	2.59[c]	1.39[ab]	1.13[a]	1.70[a]	1.35[abc]
12k	2.16[a]	1.34[a]	1.16[a]	1.65[a]	1.22[ab]
21k	3.64[e]	1.32[a]	1.17[a]	1.72[ab]	1.06[a]
50k	2.08[a]	1.47[ab]	1.18[a]	1.92[c]	1.32[abc]
125k	3.17[d]	1.57[b]	1.31[a]	1.82[abc]	1.57[e]
500k	2.97[d]	1.49[ab]	1.33[a]	1.79[abc]	1.36[bcd]
950k	2.45[bc]	1.45[ab]	1.13[a]	1.77[abc]	1.45[cde]

Note. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa. Mean values with the same letter do not differ significantly ($p > 0.05$) according to the Tukey test.

Firmness is an important physical parameter used to assess fruit quality during ripening, storage, and distribution. It determines the storability of the fruit and its suitability for transport. Loss of firmness is the most noticeable change that occurs in fruit during storage or transport [28, 29]. Kamchatka berries have a relatively low firmness [30], and this feature is also highly dependent on the cultivar [31]. Therefore, finding a substance to increase this parameter would be valuable. A popular way to protect fruit is to soak it in an agent. A CH coating could reduce the rate of softening by reducing metabolic activity because it acts as a barrier to oxygen and carbon dioxide absorption [32]. However, inaccurate drying of the fruit can result in fungal infestation.

We noted a wide variation in the firmness of the treated Kamchatka berries (Figure 2). This is due to the uneven ripening of the berries and their dark, almost black colour, which they acquire at an early stage of ripening. This makes it difficult to distinguish between ripe and still-ripening berries. The application of CH 3k, 5k, 50k, and 950k produced the greatest increase in the average firmness of the berries. In contrast, CH 3k and 50k increased the maximum firmness of Kamchatka berries (Figure 2). Low-molecular-weight CH 3k, 5k, 12k, and 50k also increased fruit/peel resistance to mechanical damage. This is very important at the fruit-harvesting stage as well as during transport. Plants sprayed with CH 3k, 5k, and especially 50k were dark in colour at harvest, indicating their ripeness (Figure 3). These are typical colour characteristics of Kamchatka berries at harvest [4].

The application of CH can increase the firmness of fruit due to the formation of a CH film on the fruit surface. Such a coating acts as an oxygen barrier, which slows down metabolic activity and oxidative decomposition of glucose, process that promote the ripening process [33]. The CH coating can also reduce cell wall degradation, which in turn maintains cell turgidity and protects the structure of the cell wall. The increase in fruit firmness may be due to the stopping/slowing of starch hydrolysis, or a reduction of the breakdown of proto-pectins into soluble pectin. CH also increases the extract content of fruit [34].

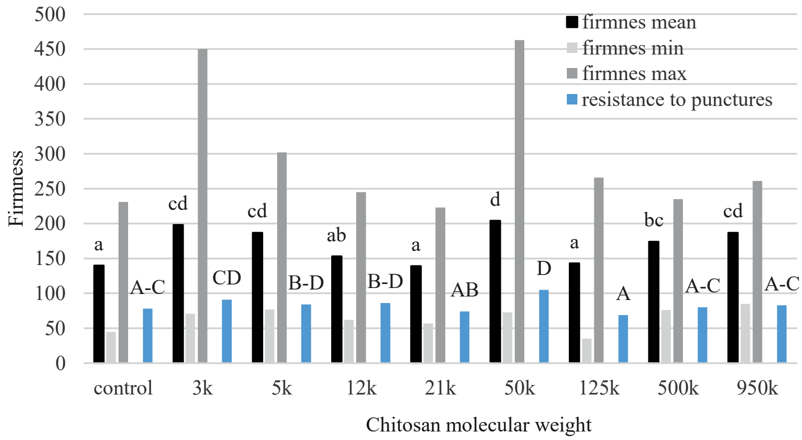

Figure 2. The effect of foliar-applied chitosan on the firmness and damage resistance of Kamchatka berries. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa.

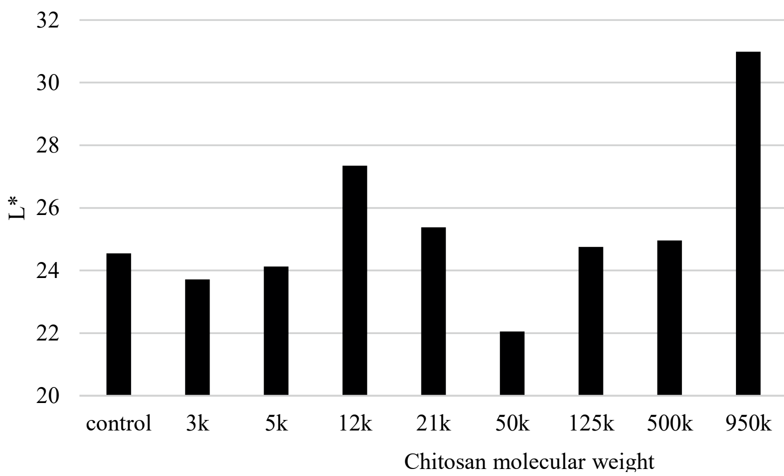

Figure 3. The effect of foliar-applied chitosan on the colour of Kamchatka berries. The molecular weights of chitosan: 3,000 (3k), 5,000 (5k), 12,000 (12k), 21,000 (21k), 50,000 (50k), 125,000 (125k), 500,000 (500k), and 950,000 (950k) kDa.

4. Conclusion

CH is an easily accessible, environmentally friendly biopolymer with great potential for use in horticulture due to its biostimulatory, elicitation, and antimicrobial activity, as well as plant growth stimulation and tolerance to environmental stresses. However, CH had inconclusive effects on the growth, yield, and fruit quality of Kamchatka berry bushes. CH with lower molecular weights (3kDa and 12kDa) stimulated plant growth, while CH with a molecular weight above 50kDa reduced plant growth compared with control shrubs.

However, there was a significant increase in the yield of plants sprayed with CH 125 kDa and 500 kDa. CH 125 kDa treatment increased fruit weight throughout the yield period. The application of CH 3, 5, 50 and 950 kDa increased the average berry firmness, while CH 3, 5, 12 and 50 kDa increased berry/peel resistance to mechanical damage. CH 3, 5 and especially 50 kDa produced berries that were dark in colour at harvest, indicating ripeness. Based on our data, we recommend higher molecular weight CH to increase yield and lower molecular weight CH to improve firmness and damage resistance.

5. References

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