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*Proceeding Paper*

# Effects of Chitosan Oligosaccharide Lactate on Growth and Overwintering of Evergreen Fern *Cyrtomium fortunei* var. *clivicola* †

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**Abstract:** Chitosan derivatives with a low molecular weight can enhance plant growth and improve tolerance to various stresses. Hardy ferns form a group of attractive garden perennials with an unknown response to plant biostimulants. Cold winter temperature limits the growth and reduces the decorative value of evergreen fern species. This study was carried out to explore the effects of chitosan oligosaccharide lactate (COL) with Mn = 5000 on the growth of the evergreen fern *Cyrtomium fortunei* var. *clivicola*. COL sprayed at 50 and 100 mg/L significantly increased the height of the plant and fresh weight of the above-ground and underground parts of *C. fortunei* var. *clivicola* compared to control plants. Moreover, ferns sprayed with 50 and 100 mg/L COL also had higher leaf total chlorophyll contents and value score. These results indicate that COL improved *C. fortunei* var. *clivicola* growth and overwintering and may be used for high-quality hardy ferns' production.

**Keywords:** hardy ferns; biostimulants; oligochitosan; low temperature stress



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## 1. Introduction

The wide demand for a plant biostimulant inspires the search for sources of innovative natural substances with a high biostimulant action. Particular attention is paid to natural polysaccharides, which are eco-friendly, biocompatible, non-toxic, reactively bioactive and inexpensive. Products of polysaccharides with a low molecular weight, and therefore with different physico-chemical properties, can affect the growth and physiological activity of plants to variable degrees [1,2]. Chitosan is an example of a widespread biostimulant obtained in the process of chitin de-N-acetylation. It has been proven that chitosan, particularly with a low molecular weight, positively affects the growth and yield quality [3], regulates physiological and metabolic processes [4], and also increases plant tolerance to abiotic stress [5,6]. Chitosan oligosaccharide with a low molecular weight obtained by the degradation of chitosan by lactate could be a promising approach in horticultural production. This product has an outstanding water solubility and high biological activity, and therefore it may be easily absorbed and utilized by plants [7].

The offer of hardy ornamental plants continues to be enriched because the novelties always enjoy interest and higher prices are obtained. Hardy ferns are perennials that are still little known and very attractive [8]. *Cyrtomium fortunei*, commonly called holly fern, is an easy-to-grow evergreen fern native to Japan, Korea and China. *Cyrtomium fortunei* var. *clivicola* differs from the species by its more compact size, with lanceolate and yellow green leaves held on nearly horizontal, arching fronds. Cold winter temperature may limit the growth and reduce the decorative value of evergreen fern species. The

available literature lacks detailed data on the effects of plant growth-regulating compounds, including biostimulants, on the growth and winter hardiness of fern.

Hence, the aim of the present study is to evaluate after winter the morphological features of *C. fortunei* var. *clivicola* plants pretreated with chitosan oligosaccharide lactate (COL) with a low molecular weight.

## 2. Materials and Methods

Two-year-old rhizomes of *Cyrtomium fortunei* var. *clivicola* were planted in 1.7 L pots and grown from summer 2020 to spring 2021 in a plastic tunnel of the Department of Horticulture, West Pomeranian University of Technology in Szczecin (53°25′ N, 14°32′ E, 25 m asl., sub-zone 7a USDA). The growing medium consisted of peat substrate (pH 6.0) added with PG Mix fertilizer at a dose of 1 g/dm$^3$. In October 2020, the plants were sprayed twice with a 50 or 100 mg/L solution of chitosan oligosaccharide lactate $(C_{12}H_{24}N_2O_9)_n$ with the number average molecular weight (Mn) = 5000. The control plants were sprayed with water. Each treatment included 20 rhizomes, planted in four replicates of 5 rhizomes. The impact of chitosan oligosaccharide lactate on plant growth was assessed after winter. In March 2021, the plant height from the ground level to its highest point, plant width at its widest point, leaf total chlorophyll contents in SPAD (Soil Plant Analysis Development) units with the Chlorophyll Meter SPAD-502 optical apparatus (Konica-Minolta Corporation, Osaka, Japan) determined from 5 points of leaves, and leaves' fresh weight of leaves and rhizomes per plant were measured. The visual score was rated on a scale of 1–5 by three autonomous investigators. The results were statistically analyzed using a one-way analysis of variance (ANOVA), and the means were compared by a Tukey's multiple comparison test.

## 3. Results and Discussion

The foliar application of COL improved all of the growth attributes of plants except for the leaves number (Table 1, Figure 1). The fern plants sprayed with COL at 50 and 100 mg/L showed a significant increase in plant height (by 25.4% and 28.5%, respectively) in comparison to controls. COL at 50 and 100 mg/L also improved the leaf total chlorophyll contents (by 30.5% and 30.1%, respectively). COL at 100 mg/L did not change the diameter of the plant. Nevertheless, COL at 50 mg/L increased the diameter of the plant by 15.3% versus untreated controls (Table 1).

**Table 1.** Effect of foliar applications of chitosan oligosaccharide lactate on the plant height, plant diameter, number of leaves per plant and leaf total chlorophyll contents (SPAD) of *Cyrtomium fortunei* var. *clivicola* plants after being overwintered in a plastic tunnel.

| Chitosan (mg/L) | Plant Height (cm) | Plant Diameter (cm) | Leaves (no.) | SPAD |
|---|---|---|---|---|
| Control (water) | 13.0 b [1] | 48.3 b | 30.3 a | 22.6 b |
| 50 | 16.3 a | 55.7 a | 28.3 a | 29.5 a |
| 100 | 16.7 a | 45.8 b | 30.8 a | 29.4 a |

[1] Mean values in each column followed by a different lowercase letter are significantly different by Tukey's least significant difference test (LSD) at $p < 0.05$.

COL applications at 50 and 100 mg/L significantly enhanced the fresh weight of leaves (by 24.8% and 27.1%, respectively) and rhizomes (by 53.4% and 14%, respectively) compared to the control. The plants sprayed with COL at 50 mg/L exhibited the greatest fresh rhizomes weight (50.3 g). COL treatments also influenced the visual score of plants after being overwintered in a plastic tunnel. The fern plants sprayed with 50 and 100 mg/L COL had a significantly greater visual score in comparison with untreated plants (Table 2).

**Figure 1.** *Cyrtomium fortunei* var. *clivicola* at six months after treatment: (**left**)—untreated control; (**right**)—50 mg/L chitosan oligosaccharide lactate.

**Table 2.** Effect of foliar applications of chitosan oligosaccharide lactate on the fresh weight of leaves and rhizomes, and the visual score of *Cyrtomium fortunei* var. *clivicola* plants after being overwintered in a plastic tunnel.

| Chitosan (mg/L) | Leaves Fresh Weight (g) | Rhizomes Fresh Weight (g) | Visual Score |
|---|---|---|---|
| Control (water) | 26.6 b [1] | 32.8 c | 3.7 b |
| 50 | 33.2 a | 50.3 a | 4.5 a |
| 100 | 33.8 a | 37.4 b | 4.9 a |

[1] Mean values in each column followed by a different lowercase letter are significantly different by Tukey's least significant difference test (LSD) at $p < 0.05$.

The obtained results indicated the stimulating effect of COL on the plant height and diameter, fresh biomass, and visual score of the evergreen fern *Cyrtomium fortunei* var. *clivicola* after winter. In addition, the plants treated with COL had a greater greenness index at six months after treatment. Numerous other studies have also reported that natural polysaccharides such as chitosan and their derivatives improved the growth attributes and enhanced the levels of chlorophylls in plants [2–4]. It can thus be supposed that chitosan improves the efficiency of water and individual elements' uptake, which in turn is associated with the intensification of plant growth [2,3,9].

## 4. Conclusions

Our report is the first to show that chitosan in the form of oligosaccharide lactate affects fern growth and physiological processes. The results described in the present work not only supplement the current state of knowledge on the impact of oligochitosan on plant growth, but can also contribute to the improvement and popularization of *Cyrtomium fortunei* var. *clivicola* cultivation and can be used in the production of other hardy ferns.

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