Seaweed (*Ascophyllum nodosum*) extraction method produces chemically different formulations with contrasting effects on turfgrass rooting

Owen, A.G., T.I. Williams and D. Hiltz

Introduction

Seaweed extracts have been applied to turfgrass surfaces, particularly golf greens, as part of a maintenance programme for many years[1]. There are numerous commercial products available to the turf manager containing seaweeds from a range of sources and utilising multiple extraction techniques. KAHN[2] comprehensively reviewed the benefits of applying seaweed extracts to plants, illustrating a wide range of potential benefits; including increased plant rooting and improved abiotic and biotic stress tolerance, important characteristics for the contemporary sports turf manager. KAHN also emphasised that seaweed extracts are complex, contain many different compounds; macro and micro nutrients, amino acids, vitamins, plant hormones and a range of simple and complex carbohydrates and the mode of action for generating the in-plant benefits are not often clear, suggesting many components act synergistically. This complexity has led to a one-size fits all approach in the marketing of commercial seaweed extracts which is false. GUINAN[3] demonstrates a clear specificity of response of plants to differing seaweed extracts and advocates a more rigorous fitness-for-purpose approach. It was the objective of this research to examine the effect of a range of extract types from the same source seaweed to turf grass rooting and to determine if this classic turf response to seaweed extract applications was comparable across extract types.

	ANE-01*	ANE-02*	ANE-03*	ANE-04~
pH	11.0	4.6	3.1	5.9
Dry Matter w/w %	9.5	2.9	7.7	10.1
Carbohydrates (%)				
Mannitol	0.475	0.667	0.847	0.606
Alginate	1.425	0.232	0.984	2.520
Fucoidins	0.891	0.232	1.540	1.010
Laminarin	0.285	0.319	0.539	0.404
Nutrients (ppm)				
Calcium	418	170	690	1520
Magnesium	504	310	920	1010
Phosphorus	105	196	1190	120
Potassium	17500	1130	2420	2200

Tab.1: Chemical analysis of *Ascophyllum nodosum* extracts. (*ANE01-03 are extracted samples, ~ANE04 is a micronized suspension).

Materials and Methods

A homogeneous sample of harvested and dried *Ascophyllum nodosum* (L.) le Jol. from Nova Scotia, Canada was extracted utilising four methods to produce samples of aqueous *Ascophyllum nodosum* extract (ANE) for comparison. A ratio of 1:7 solid:liquid with a five hour, constant stirring method was utilised for three extractions. ANE-01; Potassium hydroxide extract utilised to produce an alkaline extraction at pH 12. ANE-02; water at 70 °C utilised to produce a hot water extract. ANE-03; nitric acid / phosphoric acid at 70 °C to produce a hot acid extract at <pH 3.0. ANE-04 was developed differently but using the same ratio; water at < 10 °C added and micronized in a blender to produce a cold-blend water suspension / extract. Each extract was filtered (1 mm mesh) and centrifuged at low speed to remove seaweed debris. Each extract was chemically analysed using ICP-AES, to determine macro and micro nutrient concentrations, plus signature carbohydrates (mannitol, laminarin, fucoidins and alginate) were measured using various high-performance liquid chromatography methods based on those outlined by MANNS[4].

Rooting of seedling *Lolium perenne* (Torsion) was assessed within clear plastic phytotox kit test containers, measuring 155 mm x 210 mm x 8 mm, (Microbiotests, Gent, BE). Seeds were germinated in a petri-dish and transferred at day 7 to the test containers. Four seedlings per container were

[1] ARTHUR, J., 2003: Practical Greenkeeping. R&A. St Andrews. 312p.

[2] KAHN, W., U.P. RAYIRATH, S. SUBRAMANIAN, M.N. JITHESH, P. RAYORATH, D.M. HODGES, A.T. CRITCHLEY, J.S. CRAIGI an J. NORRIE, 2009: Seaweed extracts as biostimulants of plant growth and development. J Plant Growth Regul. 28. 386-399.

[3] GUINAN, K.J., N. SUJEETH, R.B. COPELAND, P.W. JONES, N.M. O'BRIEN, H.S. SHARMA, P.F.J. PROTEAU and J.T. O'SULLIVAN, 2013: Discrete roles for extracts of Ascophyllum nodosum in enhancing plant growth and tolerance to abiotic and biotic stresses. Acta Horticulturae 1009.15

[4] MANNS, D., A.L. DEUTSCHLE, B. SAAKE and A.S. MEYER, 2014: Methodology for quantitative determination of the carbohydrate composition of brown seaweeds (Laminariaceae). Rsc Advances, 4(49), 25736-25746.

	Total root length (cm)	Mean root diameter (mm)	Total root volume (cm^3)
Control	420 b	0.173 b	0.104 b
ANE-01	608 a	0.181 ab	0.158 a
ANE-02	340 b	0.195 a	0.102 b
ANE-03	209 b	0.186 ab	0.055 b
ANE-04	320 b	0.171 b	0.074 b

Tab. 2: Root characteristics measured by WinRhizo. Four plants per sample unit n=6. Accompanying letters indicate difference P<0.05.

transferred with the establishing root placed onto filter paper dampened with 20 ml of one of each of the four extracts diluted to equivalent field application rates (10L/500L water ha^{-1}). Six test containers were prepared for each extract alongside a control (only water applied). The seedlings in the test containers were then grown-on in a constant temperature cabinet set to provide a 16-hour light period per day at 20 °C. At day 15 test containers were scanned using a flat-bed scanner and the rooting area analysed with the WinRhizo root scanning software. Each container set of 4 plants was treated as a single experimental unit, to reduce root overlap errors. Data was collected for total root length, total root volume and root diameter.

Results and Discussion

Chemical analysis of the extracts reveals distinct differences (Table 1), particularly in terms of extract pH; from 3.1 for ANE-03 to 11.0 for ANE-01, but also for carbohydrates such as alginate (%) which shows a 10-fold difference between ANE-02 and ANE-04. Macronutrient content is generally greater for the micronized suspension ANE-04 than for the extracts, except for potassium, which shows elevated values for ANE-01 because of the KOH extracting solution. The low pH extraction increases the amount of phosphorus, but phosphoric acid was part of the extractant.

Measured rooting characteristics demonstrate significantly different values for the extracts (Table 2). ANE-01 shows significantly greater total root length, being the only extract to promote *Lolium perenne* root length significantly over the control set. Mean root diameter is significantly greater for the hot water extract when compared with the control and ANE-04. Total root volume is significantly greater for ANE-01, being the only extract to promote total root volume over the other extracts and the control.

The results demonstrate clear differences in chemical constituency between various seaweed extracting solutions and techniques from the same base seaweed. The differing pH and chemical extractants solubilize varying amounts of carbohydrates and nutrients from seaweed. Only a few characteristics were measured in this study, but this data clearly supports the supposition that different extractants and extraction methods produce differing chemical profiles of seaweed extracts for a range of constituent compounds found within the same seaweed. However, for the turf manager what is most critical is not the extract chemistry but the response in the plant following application. When we measure seedling rooting response of *Lolium perenne*, ANE-01; an alkaline extraction of the base seaweed, produces significantly greater root length and total root volume. This clearly demonstrates that differing seaweed extracts produce contrasting rooting responses in the turf grass plant, however the causal factor for this cannot be determined. It is likely that various components of the extract act synergistically and very likely that unmeasured specific compounds derived from the extracting process contribute to this effect.

Authors:

Andy G. Owen,
ICL-Specialty Fertilisers,
Waadenburg, The Netherlands,

Tamsin I. Williams,
Department of Biological Sciences,
Royal Holloway University of London,

D. Hiltz,
Acadian Seaplants Limited,
Dartmouth, Nova Scotia, Canada.