

Screening Trial to Evaluate Lumen[®], Magno[®] & Kestrel Zn[®] on Vegetative Growth and Mineral Nutrient Uptake in Barley

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Abstract

Soil enzymes are naturally occurring biological catalysts that accelerate the decomposition of soil organic matter and the cycling of essential nutrients. They break down complex organic compounds into simple organic molecules and inorganic ions that are then available as nutrients for plants and soil microorganisms. The combined effects of improved nutrient uptake, enhanced root development and increased rhizosphere microbiological activity contribute to plant vigour, increased biomass and potentially higher yields.

Soil enzymes such as Lipase, Mannanase and Phosphatase are crucial catalysts in the global cycles of carbon, nitrogen and phosphorus. Research into the potential of soil applied enzymes as a primer of soil microbiological activity, via essential nutrient availability and enhanced nutrient uptake by plants, has involved the addition of zinc as a co-factor which boosts the activity of soil-applied Lipase and Mannanase.

A bioassay pot trial was conducted with barley in a highly fertile, alluvial clay soil to evaluate the effects of these enzymes on root and shoot growth as well as on nutrient uptake. Lumen[®] (a.i. Lipase, Mannanase) and Magno[®] (a.i. Phosphatase & Mannanase) were applied with Kestrel[®] Zinc (as dipotassium EDTA) directly to forage barley seeds as a liquid seed dressing prior to planting. Results from this bioassay trial showed that the enzyme treatment enhanced germination, emergence and early root development compared to the untreated control. At harvest, dry masses (DM) of whole plants, shoots and roots were significantly greater than the control by averages of 20% (P<0.01), 17% (P<0.01) and 25% (P<0.05) respectively. Similarly, treated plants absorbed a significantly greater quantity of some mineral nutrients (i.e. grams/total dry mass/pot as opposed to grams/kg tissue concentration). Accumulation of N-P-K-Ca-Mg and N-P-K was greater in shoots (P<0.05) and roots (P<0.05) respectively.

1. Literature Review

1.1 Enzymes

Enzymes are proteins produced by living organisms that acts as catalysts to accelerate specific biochemical reactions without themselves being consumed or permanently altered by the reaction (Cooper 2000). Enzyme proteins are highly specific in that they generally catalyse the conversion of only one type (or at most a range of similar types) of substrate molecule into product molecules (Robinson 2015) (Fig. 1). A small quantity of an enzyme can catalyse a large quantity of a specific substrate. The enzyme remains unaltered at the end of the reaction and is free to bind with the substrate again and again. They ultimately biodegrade into CO₂ water, nitrogen and energy.

The number of substrate molecules that can be converted to product by a single enzyme molecule per unit time (typically per minute or per second) is known as the turnover rate/frequency/number and is expressed as a constant, K_{cat}. Turnover rates are highly variable between enzymes, for example, Carbonic Anydrase has a K_{cat} of 600,000 while Tyrosinase has a K_{cat} of 1 (Robinson 2015).



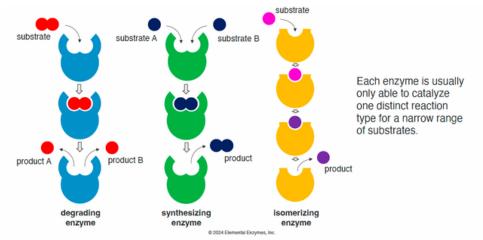


Fig. 1. Enyzme mode of action

1.1.1 Enzyme structure

Amino acid-based enzymes are globular proteins that range in size from less than 100 to more than 2 000 amino acid residues. These amino acids can be arranged as one or more polypeptide chains that are folded and bent to form a specific three-dimensional structure, incorporating a small area known as the active site, where the substrate actually binds. The active site may well involve only a small number (less than 10) of the constituent amino acids (Fig. 2).

It is the shape and charge properties of the active site that enable it to bind to a single type of substrate molecule, so that the enzyme is able to demonstrate considerable specificity in its catalytic activity (Robinson 2015).

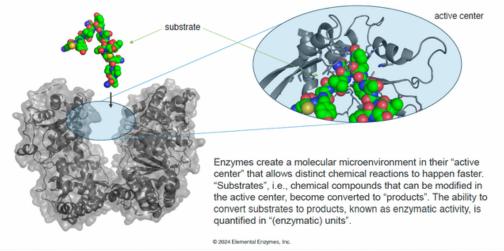


Fig. 2. Enzyme structure

1.1.2 Enzymes form complexes with their substrates

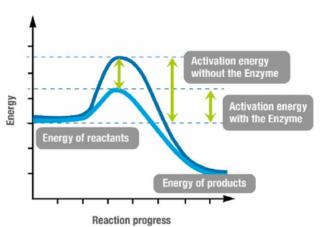
Biochemical reactions which are catalysed by enzymes are often described as proceeding through 3 stages as follows:



$E + S \rightarrow ES$ Complex $\rightarrow E + P$

The ES complex represents a position where the substrate (S) is bound to the enzyme (E) such that the reaction (whatever it might be) is made more favourable. As soon as the reaction has occurred, the product molecule (P) dissociates from the enzyme, which is then free to bind to another substrate molecule. At some point during this process the substrate is converted into an intermediate form (often called the transition state) and then into the product (Robinson 2015).

In terms of energetics, reactions can be either exergonic (releasing energy) or endergonic (consuming energy). However, even in an exergonic reaction a small amount of energy, termed the activation energy, is needed to give the reaction a 'kick start.' As shown in Figure 3, enzymes are considered to lower the activation energy of a system by making it energetically easier for the transition state to form. In the presence of an enzyme catalyst, the formation of the transition state is energetically more favourable (i.e.



lerating the rate at which the reaction will proceed, ither the reactant or the product (Robinson 2015).

Fig. 3. Effect of an enzyme on reducing the activation energy required to start a reaction.

1.2 Soil enzymes

Soil enzymes are biocatalysts produced by plant root secretion, residue decomposition, microbial metabolism, animal activities, etc. They participate in soil organic matter decomposition, nutrient cycling, and energy metabolism and have certain ecological indicators. The enhancement of soil enzyme activity can improve the effectiveness of soil nutrients, such as C, N, and P, by accelerating the degradation and conversion rates of organic and inorganic substances (Das et al. 2010, Gao et al. 2025). Enzymes break down complex organic compounds into simple molecules that are available to plants and soil microorganisms.



Nutrient cycling in soils involves biochemical, chemical, and physicochemical reactions. All biochemical reactions are catalyzed by enzymes, hence making enzymes suitable as indicators of biological activity. While soil enzyme activity holds great potential as a biological indicator of soil health it must always be considered together with other physicochemical and biological indicators of soil health (Alkorta et al 2003). Soil enzymes used as indicators of soil health have been summarised by Das and Varma (2010, Table 1) while the primary microbial enzymes in soil have been reviewed (Duanoras et al 2024, Table 2).

The addition of soil enzymes to the soil rapidly accelerates natural processes that activate the rhizosphere. They provide plant-available nutrients and simple molecules that activate the proliferation of endemic micro-organisms which continue the process. Soil applied enzymes accelerate and boost natural soil processes.

1.2.1 How soil enzymes work

Catalysis:

Soil enzymes act as biological catalysts, speeding up the chemical reactions that transform unavailable complex organic compounds into simpler molecules that plants and soil micro-organisms can utilise. Individual enzymes react in a defined process with a specific substrate. They are active at low applied rates.

Table 1. Soil enzymes as indicators of soil health

Soil enzyme	Enzyme reaction	Indicator of microbial activity				
Dehydrogenase	Electron transport system	C-cycling				
b-glucosidase	Cellobiose hydrolysis	C-cycling				
Cellulase	Cellulose hydrolysis	C-cycling				
Phenol oxidase	Lignin hydrolysis	C-cycling				
Urease	Urea hydrolysis	N-cycling				
Amidase	N-mineralization	N-cycling				
Phosphatase	Release of PO4	P-cycling				
Arylsulphatase	Release of SO4	S-cycling				
Soil enzymes	Hydrolysis	General organic matter degradative enzyme activities				



Table 2. Microbial enzymes in soil

Soil enzyme	Enzyme reaction	Role in the soil				
β-1,4-Glucosidase	Cellulose decomposition, soil organic carbon (SOC) hydrolysis	C-acquisition				
β(1-3) Glucanase	C cycle, production of reactive oxygen species (ROS)	C-acquisition				
Dehydrogenases	Oxidation of soil organic matter	C-acquisition				
Cellobiohydrolase/exo- and endocellulases	C cycling; cellulose decomposition; SOC hydrolysis	C-acquisition				
Invertase	C cycle; hydrolysis of sucrose	C-acquisition				
β-1,4-N-acetyl-glucosaminidase	C & N cycles; chitin degradation; SOC hydrolysis	C- & N-acquisition				
Nitrate reductase	N cycling; C sequestration	C- & N-acquisition				
Phenol oxidases	Oxidation of polyphenols	C- & N-acquisition				
Peroxidases	Oxidation of polyphenols	C- & N-acquisition				
Leucine aminopeptidase	Hydrolysis of leucine residues at the N-terminus of peptides and proteins	N-acquisition				
Ureases	N cycling; hydrolyses N compounds to NH4	N-acquisition				
Acid/alkaline phosphatases	P cycling; mineralisation of organic P; transformation of P from organic to	P-acquisition				
Phytases	P cycling	P-acquisition				
Arylsulphatase	S cycling	S-acquisition				
Esterases	Detoxification of lipidic pollutants	Detoxification of lipidic pollutants				
Lipases	Detoxification of lipidic pollutants	Detoxification of lipidic pollutants				
Catalases	Oxidoreductase associated with aerobic microbial activity					

Organic matter decomposition:

They break down soil organic matter including plant, animal and microbiological residues.

Nutrient cycling:

Decomposition by enzymes recycles essential nutrients making them available to plants and soil microorganisms.



Microbial activity:

Soil applied enzymes provide nutrients and stimulate dormant soil microorganisms in the rhizosphere, priming microbial activity that continues to supply enzymes and provide soil organic matter decomposition and nutrient cycling.

1.3 Elemental Enzymes

1.3.1 Mannanase

Mannans are the major constituents of the hemicellulose fraction in softwoods and show widespread distribution in plant tissues. The mannanases are known to be produced by a variety of bacteria, fungi, actinomycetes, plants and animals (Chauhan et al 2012). Mannanases breaks down specific mannans in plant cell walls and root exudates and release nutrients and simple sugars that benefit microbial activity, support plant root growth, plant nutrient availability and nutrient absorption.

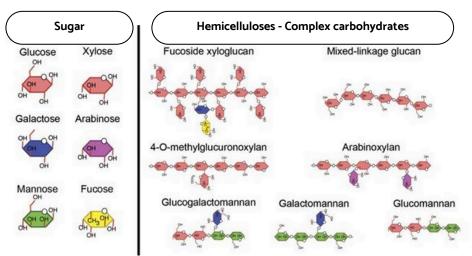


Fig. 4. Mannanase enzyme activity, substrates and products

1.3.1.1 How Mannanase works in the soil

Breakdown of root exudates:

Mannanase enzyme specifically catalyses the breakdown of mannans, which are complex carbohydrates found in exudates surrounding plant roots, into simple sugars.

Release of nutrients and sugars:

The enzyme catalyses the release of sugars that provide energy to soil micro-organisms. In the rhizosphere it also facilitates the release of nutrients tied up in soil organic matter and those present in the soil, making them more available to the plant. Resulting in increased uptake. Increased activity of soil micro-organisms contributes enzymes that continue the process.

Enhanced soil structure:

Mananase interacts, in the rhizosphere, with soil micro-organisms and plant roots. This interaction creates a favourable environment for root development and microbial activity, improving soil conditions. The combined effects of improved nutrient uptake and root development contribute to increased growth and potentially higher yields.



1.3.2 Lipase

Lipase is a naturally occurring soil enzyme that degrades soil phospholipids.

How lipase works in the soil:

Lipase catalyses the breakdown of lipids in soil organic matter, mucilage and root exudates. Lipase catalyses the cleavage of phosphatidylcholine into a choline phosphate group and diacylglycerol, a biological surfactant. Root access to phosphate is enhanced along with microbial activity in response to increased phosphate availability. Lipids in mucilage can reduce rates of both nitrate conversion and ammonium absorption, limiting the potential uptake of nitrogen by plants. The breakdown of lipids in the rhizosphere may enhance plant uptake of nitrogen.

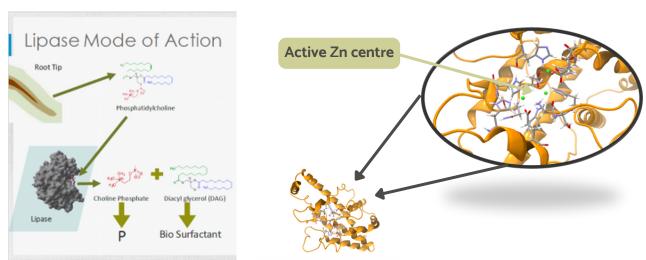


Fig. 5. Lipase structure, active centre, Zn co-factor and mode of action

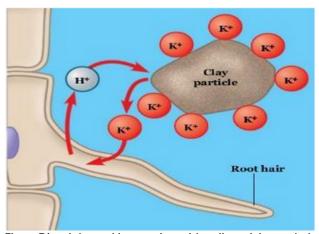


Fig. 6. Diacylglycerol interaction with soil particles and plant roots

Diacylglycerol (DAG) is a biological surfactant that provides energy to support phosphatase catalysed release of phosphate. It assists release of phosphate and potassium ions from clay particles near developing roots.



1.3.3 Phosphatase

Phosphatase enzymes catalyse the hydrolysis of organic molecules containing phosphorus. Phosphatase enzyme breaks ester bonds to cleave the phosphate group, producing soluble inorganic phosphate ions $(H_2PO_4^-)$ and $H_2PO_4^-$.

How Phosphatase works in the soil:

Phosphatase enzymes in soil break down organic phosphorus compounds into soluble inorganic phosphate ions. Phosphate ions are essential for plant and microbial uptake and growth. These enzymes, naturally produced by plants and microorganisms, are a critical catalyst for the biological mechanisms responsible for phosphorus cycling.

Agricultural practices significantly influence phosphatase activity which is highly important for agricultural productivity, nutrient cycling and soil health. Phosphatase enzymes often show low substrate specificity, allowing them to release inorganic phosphate from a variety of organic phosphorus substrates found in soil.

Soil organic phosphorus substrates include:

Phytate – a phosphorus storage molecule synthesised by plants and a wide range of organisms.

Phosphatidic acid - a breakdown product of cell membrane phospholipids.

Nucleotides - components of nucleic acids, RNA, DNA.

Sugar phosphates - intermediates in cell metabolism.

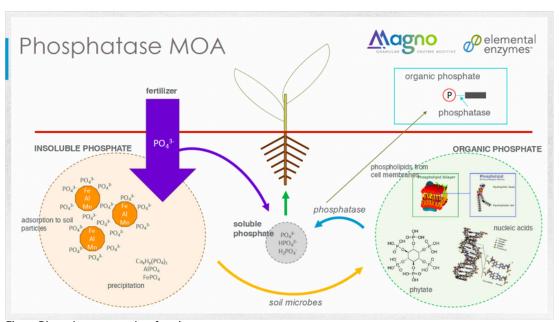


Fig. 7. Phosphatase mode of action

Soil enzymes are applied to the rhizosphere in various ways. Low per hectare application rates enable application by coating of granular fertiliser, seed dressing, in furrow at planting, with liquid fertiliser or through micro and drip irrigation.



2. Methodology

A bioassay pot trial was conducted at Toowoomba with Feed Barley var. Trojan using a fertile, alluvial clay soil sourced from Rugby Farm, Robinson Rd, Gatton. Soil was sampled on 26/03/2025 from newly-formed fertilised beds ready for lettuce production (prior to application of pre-emergent herbicide), GPS 27° 33' 53.8 S, 152° 15' 59.8 E. The previous crop was sweetcorn and the soil had a low previous crop residue and high fertility (see CSBP soil analysis below). Phosphate buffer index (PBI) of the soil was 154.8, which is considered high.

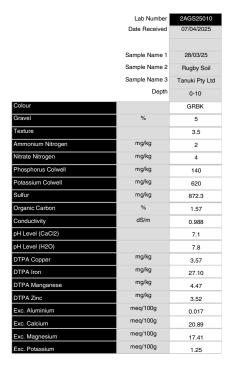
Round, black plastic pots were filled with moist soil for experimentation. Pots were 180 mm wide x 170 mm high, had a surface area of 250 cm² and a volume of 4.3 L.

Pots were filled with soil, whereafter soil in the filled pots was treated with additional preplant fertiliser prior to seeding. Thrive soluble fertiliser - 10 g / 4 L - was applied at 100 ml per pot to provide the equivalent of 25 Kg N, 5 Kg P and 9 Kg K / ha.



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	Lab Number	2AGS25010
Exc. Sodium	meq/100g	1.92
Boron Hot CaCl2	mg/kg	1.89
PBI		154.8

CSBP Lab. Extract Value

CSBP Lab. Extract Value

Thrive	N	Р	К	Mg	Fe	В	Cu	Zn	Мо
%, ww	25	5	9	0.5	0.18	0.005	0.005	0.004	0.001

2.1 Treatments & experimental design

The experiment comprised two treatments viz. (1) Enzyme + Zn treatment (T) and (2) control (C).



2.1.1 Enzyme + Zn treatment (T)

Barley seed was coated with a combination of Lumen®, Magno® and Kestrel® Zinc at a rate of 4 L / MT seed prior to seeding. Treatment rates of individual constituents were as follows:

- Kestrel Zinc (Zn as dipotassium EDTA 9.0% w/v & Potassium K as acetate 9.0% w/v) 318 ml/Ha (Zn 28.62 g/Ha)
- Lumen 25 ml/Ha
- Magno 25 g/Ha
- Balance water

Lumen (Lipase >2500 μ U/ml, Mannanase >175 U/ml, <0.01% reaction mass of 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one.

Magno (Phosphatase >250 mU/g, Mannanase >1.25 kU/g, <0.01% reaction mass of 5-chloro-2-methyl-4-iso-thiazolin-3-one, <0.1% 1,2-Benzisothiazol-3(2H)-on

2.1.2 Control (C)

Barley seed was coated prior to seeding with 4 L of water per tonne of seed.

A paired-comparison design was used so that neither treatment was biased by environmental effects.

Block 1	Block 2	Block 3	Block 4	Block 5	Block 6	Block 7	Block 8	Block 9	Block10
С	Т	С	Т	С	Т	С	Т	С	Т
Т	С	Т	С	Т	С	Т	С	Т	С

2.2 Seeding rate, watering etc.

Barley seeding rate - 80 kg/Ha aimed to achieve 150 plants/m² Eight (8) seeds were planted per pot on the 26/03/2025. Post emergence, plants were thinned to 5 plants per pot (150 plants/m²).



Pots were watered frequently (nearly daily) with a measured, volumetric application to ensure that all pots had sufficient moisture to support growth and minimise moisture stress.

2.3 Evaluation of germination and early growth

Germination rates as well as root and shoot growth of plants removed during thinning were compared visually and photographed.



2.4 Harvest and measurement of dry weights

Harvest of whole pot individual replicates, both treated and control pots, took place on 09-11/06/2025 (80 days after seeding). Soil was washed from roots to recover root mass. Shoots were separated from roots and retained as individual shoot and root replicates. Shoot and root samples were dehydrated for 24 hrs @ 70°C whereafter dry mass (DM) was recorded for individual treatment replicates. Samples were prepared and sent to CSBP Laboratory for tissue analysis.

2.5 Calculation of nutrient absorption by plants

Total nutrient absorption by plants was determined from the product of tissue nutrient concentrations (from tissue analysis done by CSBP) and dry mass i.e. nutrient concentrations of each sample were multiplied by the dry mass of each sample - for roots or shoots - to determine total nutrient removal per pot.

2.6 Statistical analysis

Dry weights and nutrient contents were all analysed with the non-parametric, Mann Whitney U test (one-tail test) to test for differences between treated and control plants.

3. Results & Discussion

3.1 Germination and early growth

Pots with treated seeds had increased germination counts and germinated and emerged earlier than plants in control pots (Fig 8). Early growth of treated plants was visibly enhanced and root-systems were more developed than controls (Fig 9).

3.2 Visual comparison at harvest

At harvest, treated plants displayed visibly greater shoot and root growth than control plants as well as different root architecture (Fig 10).

3.3 Dry weights

Dry weights of whole plants, shoots and roots are given in Graph 1, 2 and 3 respectively and show that weights of treated plants were significantly greater than control plants. Mean (average) percentage increases in dry mass (DM) and significance levels for treatment differences are as follows:

Whole plant dry mass: 20% increase (P<0.01 – highly significant)

Shoot dry mass: 17% increase (P<0.01 - highly significant)

Root dry mass: 25% increase (P<0.05 - significant)



These results show a clear biomass response to treatment with a combination of Lumen[®], Magno[®] and Kestrel[®] Zn. These results are particularly significant in that the experiment was conducted with a highly fertile soil (ECEC = 39.57) which was also well fertilised prior to experimentation. Growth responses to additional, non-fertiliser treatments may be considered unlikely under such fertile conditions although it could be contended that the higher organic matter content of fertile soils provides more substrate (than sandier, less fertile soils) available to enzymes to convert into simple sugars and mineral nutrients, thus augmenting the environment for microbial proliferation and release of additional nutrients for plant uptake.



Fig 8. Increased seedling emergence and vigour, evident prior to thinning.

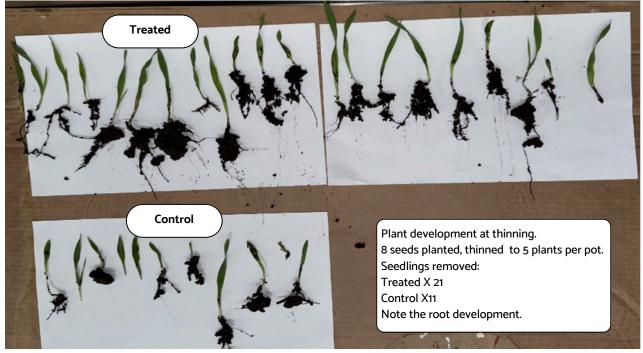
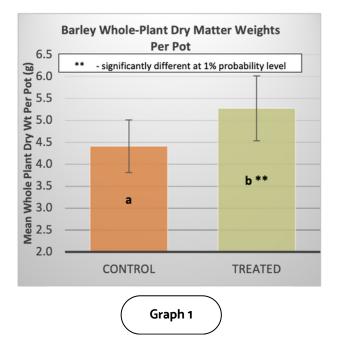


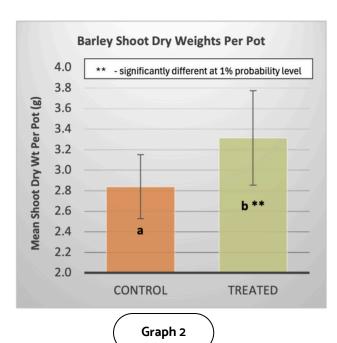
Fig 9. Seedlings removed at thinning. Treated plants showed increased growth and more extensive root systems than control plants.

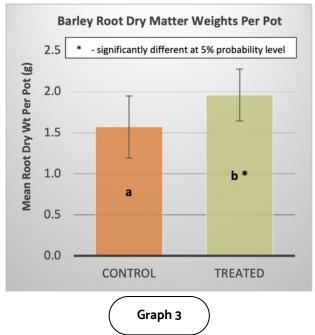




Fig 10. Increased shoot and root biomass with different root architecture.









3.4 Mineral nutrient absorption

Tissue concentrations of most mineral nutrients were not significantly different for roots or shoots of treated and control plants (Table 3). Exceptions were B in roots and Cu in roots and shoots which were significantly lower in treated plants (P<0.05). By contrast, in respect of total quantities of nutrients absorbed by all plants per pot (Table 4), roots of treated plants accumulated significantly greater amounts of N, P and K (P<0.05) while shoots had significantly greater N, P, K, Ca and Mg (P<0.05). These increases in absolute amounts of nutrients accumulated were consistent with increases in biomass of roots and shoots for treated plants and suggest that a greater root-volume enabled treated plants to explore a greater soil volume and absorb more nutrients. However, the root-volume in itself is attributable to the treatment applied and it is likely that the treatment improved nutrient availability - as would be expected from the function of theses enzymes - as well as provided an environment more conducive to or promotional of overall plant-growth.



Table 3. Mineral Nutrients - Quantities absorbed per kg dry matter

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Iron	Boron	Copper	Manganese	Sodium	Zinc
	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
BARLEY ROOTS												
Mean - Control	8550	4160	9750	2120	2840	1200	4362	3.2	82.6	109.5	1520.0	56.1
St. Dev Control	1204	357	1422	346	470	105	2318	0.3	9.4	54.8	239.4	10.6
Mean - Treated	8360	4020	10520	2130	2910	1170	5186	2.7	65.2	124.3	1470.0	53.3
St. Dev Treated	776	368	1520	374	390	95	1937	0.4	12.9	42.8	245.2	9.8
Mann-Whitney U Test Results	NS	NS	NS	NS	NS	NS	NS	SD, p<0.05	SD, p<0.05	NS	NS	NS
BARLEY SHOOTS												
Mean - Control	9660	4550	15610	3210	2940	2060	343	4.4	10.3	51.3	2570.0	35.8
St. Dev Control	582	430	1034	360	263	201	63	0.7	2.7	9.2	271.0	4.7
Mean - Treated	9660	4820	16000	3440	2940	1930	330	4.5	7.5	49.7	2620.0	31.2
St. Dev Treated	1078	429	1861	347	237	170	91	0.5	1.4	7.4	215.0	9.4
Mann-Whitney U Test Results	NS	NS	NS	NS	NS	NS	NS	NS	SD, p<0.05	NS	NS	NS

Key: NS – not significant, SD, p<0.05 – significantly different at the 5% probability level

Table 4. Mineral Nutrients - Total quantities absorbed by all plants per pot

	Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sulfur	Iron	Boron	Copper	Manganese	Sodium	Zinc
	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg	mg
BARLEY ROOTS												
Mean - Control	13.3	6.5	15.5	3.4	4.6	1.9	7.3	0.005	0.13	0.18	2.38	0.09
St. Dev Control	3.1	1.6	4.9	1.2	1.7	0.4	5.6	0.001	0.04	0.13	0.65	0.03
Mean - Treated	16.5	7.9	20.5	4.2	5.8	2.3	10.6	0.005	0.13	0.25	2.86	0.11
St. Dev Treated	3.4	1.4	3.7	1.2	1.5	0.3	5.2	0.002	0.04	0.12	0.52	0.03
Mann-Whitney U Test Results	SD, p<0.05	SD, p<0.05	SD, p<0.05	NS	NS	NS	NS	NS	NS	NS	NS	NS
BARLEY SHOOTS												
Mean - Control	27.3	12.9	44.3	9.1	8.3	5.8	1.0	0.012	0.03	0.14	7.34	0.10
St. Dev Control	2.2	1.9	5.6	1.3	0.9	0.5	0.2	0.002	0.01	0.02	1.45	0.01
Mean - Treated	31.8	16.0	53.2	11.4	9.7	6.4	1.1	0.015	0.02	0.16	8.68	0.10
St. Dev Treated	4.2	2.9	10.5	2.0	1.3	0.9	0.3	0.003	0.00	0.02	1.40	0.02
Mann-Whitney U Test Results	SD, p<0.05	NS	NS	NS	NS	NS	SD, p<0.05	NS				

Key: NS – not significant, SD, p<0.05 – significantly different at the 5% probability level



4. Conclusion

The promotion of both below-ground and above-ground growth by the combination of Lumen®, Magno® and Kestrel® Zn is significant from at least two perspectives. First, it is often difficult to obtain crop responses to the application of non-nutritional products e.g. biostimulants, plant-growth promoting bacteria etc. Second, it is usually more difficult to obtain responses to such products in well-buffered, fertile soils such as the alluvial clay soil used in this investigation. Responses obtained with this soil at very low rates of application are testament to the profound effect of this product combination on nutrient release and plant growth. Moreover, the greater absorption of mineral nutrients in treated plants illustrates the effect thereof on improving nutrient-use efficiency. A greater root-volume would also be expected to have improved water absorption, an aspect which is suggested by the improved uptake of Ca, as Ca is absorbed in mass-flow of water into plants. Finally, it should be emphasised that the greater nutrient absorption by treated plants in this trial does not support a reduction in fertiliser rates. Rather, the implication is that greater rates of fertilisation may be required under field conditions in subsequent seasons to replace nutrients absorbed, although this should be evaluated through routine soil and tissue analyses.

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LUMEN and MAGNO are products of Elemental Enzymes Inc. Kestrel Zinc is a product of TANUKI PTY LTD



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