Final Report

Micronutrient Fortification

Fortification of potato (*Solanum tuberosum*) using metal oxide nanoparticles

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Report Authors: Karen Davies

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AHDB Potatoes is a division of the Agriculture and Horticulture Development Board.

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1. SUMMARY

1.1. Aim

The project aimed to increase the phytoavailability of calcium (Ca), iron (Fe) and zinc (Zn) in order to fortify tubers for human consumption, and potentially reduce disease occurrence (soft rot) in tubers.

1.2. Methodology

Mineral uptake was observed initially in hydroponic systems, transferring to greenhouse and polytunnel conditions, with field trials utilising the iron oxide nanoparticle. Growth rates, tuber number, size and fresh weight were recorded along with dry mass analysis with mineral content of tubers and the retention of the metal oxide nanoparticle (MONP) in the soil via ICP-OES and radioactive isotope tagging using ^{59}Fe.

The suppression of bacterial transfer into the tuber at storage was conducted with calcium and iron oxide nanoparticles using methods developed at Sutton Bridge Crop Storage Research.

1.3. Key findings

- Increase in mineral content of tubers from skin to pith in all applications.
- Increased foliar growth rate and number of tubers >30mmm
- Iron increased the consistency of tuber size
- Retention of minerals in growth media, decreasing leaching and increasing phytoavailability.

2. INTRODUCTION

2.1. Nanotechnology as a sustainable mineral application

Research with the use of nanoscale science and technology, enables the characterisation and manipulation of synthesised structures [1]. Particles measuring less than 100 nm in one dimension are classed as nanoparticles (NP) [4,5,6,7], figure 1 [8].

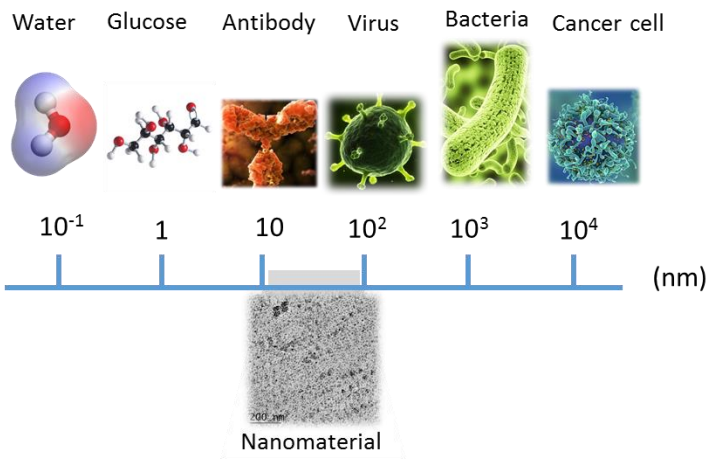

Figure 1: Schematic comparing the nm range. Adapted from Amin et al (2014) [8]

One nanometre is one millionth of a millimetre [9]. Nanoparticles (NP) are found in the natural environment in forms of dust particulates, volcanic ash, some pollens and antibodies. Due to the nano size, particles properties have been investigated for centuries in the ability to improve function, performance and increase cost-effectiveness in engineered materials resulting in an extremely diverse research field [2,5,6].

The work herein focused on the biofortification of plants using Ca, Fe and Zn minerals. Generally, plants require at least fourteen mineral elements to maintain growth and production of crops [10,11]. A depletion in phytoavailable elements results in deficiency, consequently reducing plant growth, yields and increases the plants susceptibility for disease. If the crop is deficient in a mineral, this will pass onto the consumer [12,13], causing micronutrient malnutrition (MNM) [14]. The mineral elements most commonly lacking in human diets are Fe, Zn, I, Se, Ca, Mg and Cu [4,17]. Mineral nutrition in humans is defined as the process by which substances in foods are transformed into body tissues and provide energy for the full range of physical and mental activities that make up human life [5,6].

Current agronomic strategies rely on mineral fertiliser application to increase the mineral content in edible tissues of the crop with increasing focus on the stabilisation and phytoavailability of the mineral [3,4,23].The novel application of the metal oxide nanoparticle coated with the amino acid histidine (MONP+His) as a form of fortification, hypotheses that the size of the NP can penetrate through the cell wall pores (5 to 20 nm) [24,25,26] allowing nanoparticles and nanoagregates less than the pore size to pass passively into the plant without chelation [26]. The histidine coating of the nanoparticles, increases mobility through the strata due to the ability to suspend the nanoparticle and move with water. This allows passive diffusion into the tuber membrane through a concentration gradient.

The metal oxide nanoparticle coated with the amino acid histidine (MONP+His) is a sustainable application of mineral fortification, due to the increase in retention capabilities in the soil strata over conventional metal salts and chelates, consequently decreasing the requirement for repeated applications and having a positive economic impact.

An additional benefit of MONP+His application is the coating of amino acid, histidine. Sánchez, *et al.* (2005) [27] reported that the use of amino acids in nutrient solutions improves Fe uptake by crops [27]. The presence of the amino acid increases the efficiency of nitrogen assimilation [28], in turn increasing the metabolism of the plant and accumulation of other minerals present in the soil or fertiliser [29]. Amino acids have highly diverse and essential roles in plants, by being the building blocks for enzymes and proteins, they provide important components for plant metabolism and structure [30,31], therefore providing an additional benefit to the application of MONP+His.

2.2. **Propagation and mineral composition of potato.**

The potato plant has a short life span ranging from 80 to 150 days from planting to maturity, with variation between varieties [32,36]. Its developmental stages are often described in terms of tuber initiation and growth followed by a period of dormancy and finally sprouting resulting in the next (vegetative) generation [36,37], figure 5.

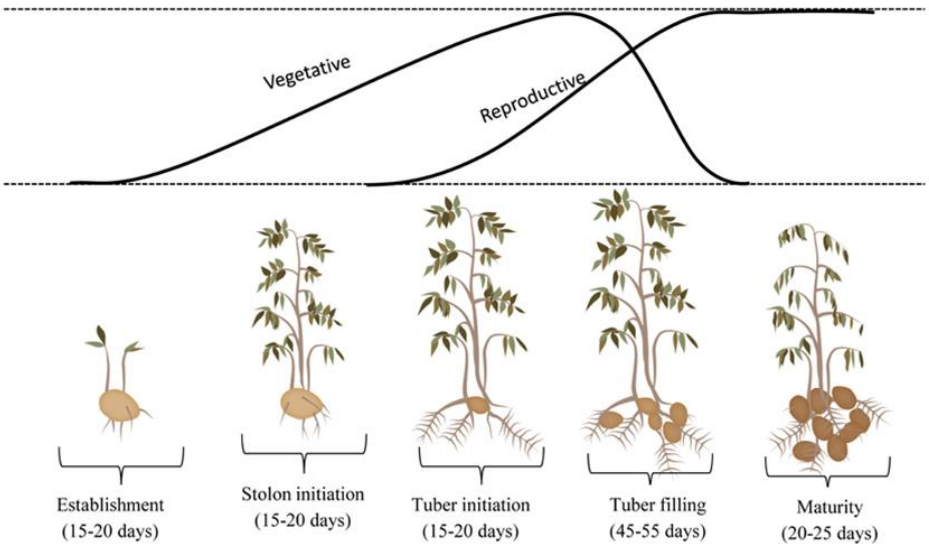

Figure 2: Generic growth cycle of potato. Adapted from Obidiegwu et al. (2015)[38]

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A 'drench' application could be applied at planting, whereby a highly concentrated solution of MONP is applied in the soil surrounding the seed potato. When the conditions are favourable for tuber initiation, the elongation of the stolon stops, and cells located in the pith and the cortex of the apical region of the stolon first enlarge and then later divide longitudinally [33,37,39]. FeNP+His. application at this stage could benefit chlorophyll production and growth with addition Fe and the assimilation of N from the presence of His.

During enlargement, tubers become the largest sink of the potato plant storing carbohydrates (mainly starch) and also significant amounts of protein [37,39]. MONP+His. would benefit the loading of potatoes and assist in the fortification of the tubers for human consumption and plant / crop development.

2.3. **Do we need fortification in our soil?**

The uptake of mineral elements by plant roots/tubers and their subsequent distribution within the plant have been the subject of studies for many decades [4,10,11,106,107,108,109]. There are several barriers that impede mineral uptake and not just the phytoavailability of minerals at the root to soil, or tuber to soil interface (i.e. rhizosphere). Free metal ions that are released via weathering of parent material, decomposition of organic matter or added via fertiliser [12,86,92,94], the ions interact with the charged particulates that may form weak complexes through cation exchange or strong bond through ligand exchange. Elements may precipitate immediately or remain in a solution depending on the ionic potential [92]. The associations these ions form largely depends on the nature of the ion and absorbing surface [92]. Metal ions of calcium, iron and zinc (Ca^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}) are taken up by the root system in a solution form [95], are unavailable as they form strong bonds with clay and organic matter in the form of oxides and hydroxides binding the metals into the soil / compost matrix [95]. Insoluble complexes are unable to move through the matrix to the rhizosphere where reduction in the pH enables chelation and uptake.

The mobility of metals within the soil are conditions are influenced by a number of factors; irrigation (via precipitation or application), pH (varying from 4.0-9.0, exerting a strong influence over free ion concentration) [97,110], CO_2, temperature, organic matter content, microorganism activity, metal species present and aeration [2,46]. Species and variety of

crop also determines mineral acquisition as the complex nature of the highly regulated homeostasis governing metal absorption, translocation within the plant, regulating transportation and redistribution (thus control over the prevention of toxic accumulation) may impede accumulation [2]. Interactions between the mineral cations and anions are rare but there is influence indirectly through membrane potential, protein electrical gradient or via feedback regulation through the rate of plant growth or metabolism [10].

Ca, as with many elements is abundant in the parent rocks of the soil, however a majority of Ca compounds are insoluble, reducing mobility in the soil and consequently to the root system of the plant [22]. Ca^{2+} is a large divalent cation in contrast to Fe and Zn ions [14] and moves in conjunction with water when free, however, this a rare occurrence as it forms a tight bond with particulates so much that Ca leaching through the soils strata does not normally occur [14,93]. Unlike other minerals such as Fe and Zn, Ca^{2+} passively diffuses into the root / tuber via a gradient caused by transpiration in the leaves [12,14,71]. Ca is less mobile in the plant and is retained in the root or tuber upon acquisition [12,97]. The xylem delivers Ca^{2+} to transpiring leaf tissues, where it is taken up from the apoplast by specific cell types [14]. Translocation of Ca^{2+} to non-transpiring or xylem-deficient tissues, occurs via the phloem [71].

Fe is essential nutrient to photosynthetic organisms as it has numerous metabolic functions and functions as a co-factor in photosynthetic and electron transport chains [111]. There are two strategies for Fe uptake known as strategy I and strategy II. Both employ an up-regulation under Fe deficiency to increase Fe availability. Strategy I, used by dicotyledons and non-grass monocotyledons, yeast and most algae [12,111,112], thus including the potato, tomatoes and chillies. The acidification by the release of organic acids and phenolic compounds, increase the concentration of Fe^{3+} in the soil solution, further chelated to Fe^{2+} by ferric reductase, which is taken up by an iron transporter [55,113].

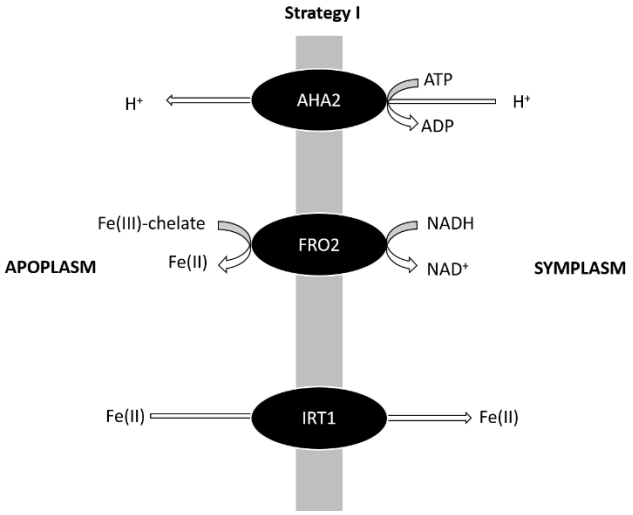


Figure 3: Strategy I uptake of iron as used by potato plants. Adapted from La Fontaine et al. (2002) [113]

Strategy II including grasses, microalgae and cyanobacteria. Mugineic acid family phytosiderophores bind Fe^{3+} in the rhizosphere which is recognised by the plant and thus taken up as well as Fe^{2+} [113]. Strategy II increases the efficiency of Fe uptake compared to strategy I, allowing grass species to grow in areas of Fe-deficiency [14].

Free Zn ions are bound in the soils matrix similarly to Fe [114] and thus highly dependent in the pH of the growth media. Normality the Zn content of non-polluted soils is approximately 3 x 10^{-8} – 5 x 10^{-7} M [29] with 15 – 30 % as free ions. Zn acts similarly to Fe ions with release in the rhizosphere due to decrease of pH are a result of proton pump [28], figure 8. Zinc is taken up as Zn^{2+} or Zn-phytosiderophore complexes across the plasma membranes of the root membranes from the rhizosphere [115]. It is commonly assumed to be transported across the root to the xylem [116,117]. As with Fe uptake, ZIP family of IRT1 (Iron-regulated transporter) [82].

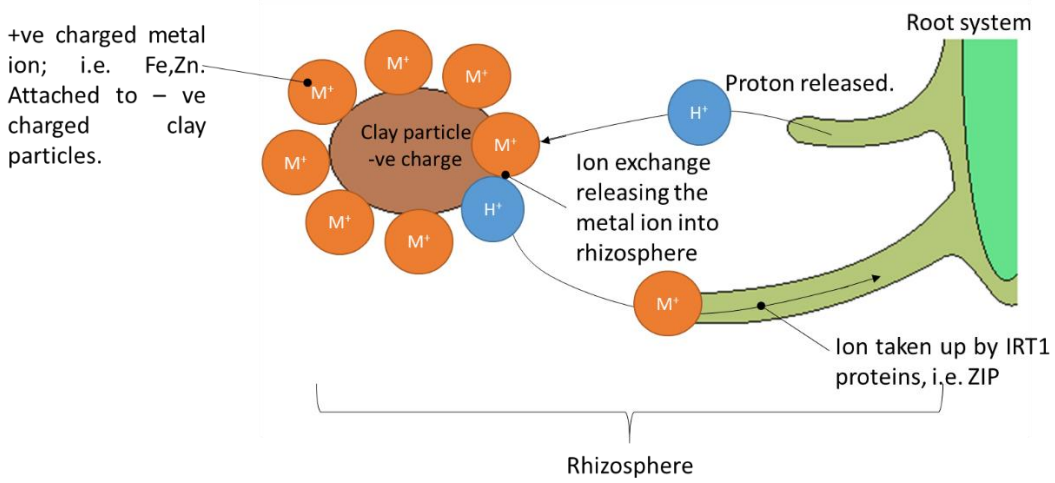


Figure 4: Simplified proton pump mechanism

Due to the low mobility of Fe in soils due to the nature of Fe being readily oxidised to form salts and highly insoluble oxides and hydroxides as follows [45]:

$$Fe^{3+} + 3(OH)^{-} \leftrightharpoons Fe(OH)_{3\ (solid)}$$

Manly Fe applications use salts, such as $FeSO_4.7H_2O$ and Fe-chelates to increase soluble Fe and hence the availability to plants particularly in calcareous conditions. Salts are extremely soluble and easily leached through the soil [45], therefore only used as a sort-term delivery. Chelates have been used since the early 1950's, as they have a high affinity constant to form a highly stable complex, delivering Fe at a reduced rate than $FeSO_4.7H_2O$ [45,46,47,48].

Ethylenediamineteraacetic acid (EDTA) is a potentially hexadentate chelating ligand (figure 10) [47,49] with each N contains a free pair of electrons and the molecule possesses four acidic hydrogens [47,49]. Other chelating agents include HEDTA, 2-hydroxyethylenediaminetriacetic acid; DTPA, diethylenetriaminepentacctic acid; EDDSA, ethylenediaminediscuccinic acid and IDSA, iminodisuccinic acid that are applied either as a foliar or root solution to increase Fe availability [50]. EDTA along with other chelates are used as a metal 'stripping agents', in the form of a treatment method to remove heavy metals from water courses due to its rapid strong chemical bond [51].

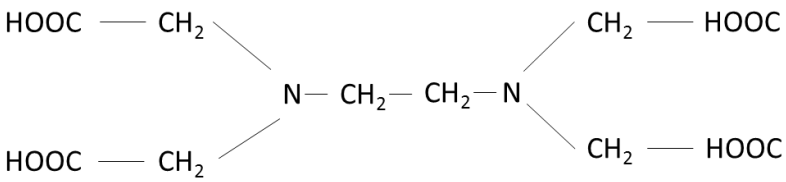


Figure 5: schematic of the structure of ethylenediaminetetraacetic acid (H_4EDTA)

Published data from Shenker and Chen (2005) [52] observed Fe-EDTA had an increased stability constant (K_{app}) above other Fe-chelates, table 3 [53], especially for Fe^{2+} is the most commonly used chelating agent. However, 81% of soil applied Fe-EDTA has been shown to leach and lost the surrounding environment, rendering the availability of Fe as poor [53].

Fe-Chelate	Log K_{app}	
	Fe^{2+}	Fe^{3+}
EDTA	22.3	11.4
HEDTA	20.3	9.5
EDDHA	24.9	5.3

Table 1: Adapted from Shenker and Chen, 2005 [53]; comparison of Fe-chelates and stability constant (K_{app}).

Although the Fe uptake mechanism, strategy I, involves the chelation of Fe^{2+} to enable transportation thought the plant and to avoid cellular damage from oxygenation damage of Fe^{2+} [54,55], this as a remedial ligand that is not as tightly bound as EDTA, therefore can be precipitated at the target site [54].

The FeNP consists of Fe^{3+} and Fe^{2+} in a stoichiometric ratio of 2:1 (Fe^{3+}/Fe^{2+}) [56] allowing a duel delivery of Fe that is phytoavailable immediately (Fe^{2+}) and a more stable Fe supply (Fe^{3+}) [4]. The amino acid coating prevents the formation of insoluble complexes with retention in the growth media to allow slow delivery of bioavailable iron.

3. MATERIALS AND METHODS

Metal oxide nanoparticles of calcium, iron and zinc oxides [55-60] where synthesised utilising patented technologies that increased the production of NP while retaining particle uniformity and the ability to form a suspension (GB2015/15000.6, GB2014/00212.5 and WO2013/136082). Coating with amino acid histidine enabled the suspension of the metal oxide nanoparticle. Analysis of size and composition was confirmed by TEM and XRD.

3.1. Hydroponic propagation (Hydro)

Initial propagation was carried out in drip feed Wilma hydroponic system (figure 11) to eliminate weather extremes and pests. Diurnal length of 12 hours using a full range sodium lamp, with a feed / watering regime of 6 hours fed, with alternate 6 hours without feed was implicated. The hydroponic nutrient feed was adapted from Wheeler, 2006 [61] with addition of the relevant MONP and concentration. The hydroponic system observed growth rate and mineral uptake to establish the optimum concentration of FeNP+His.

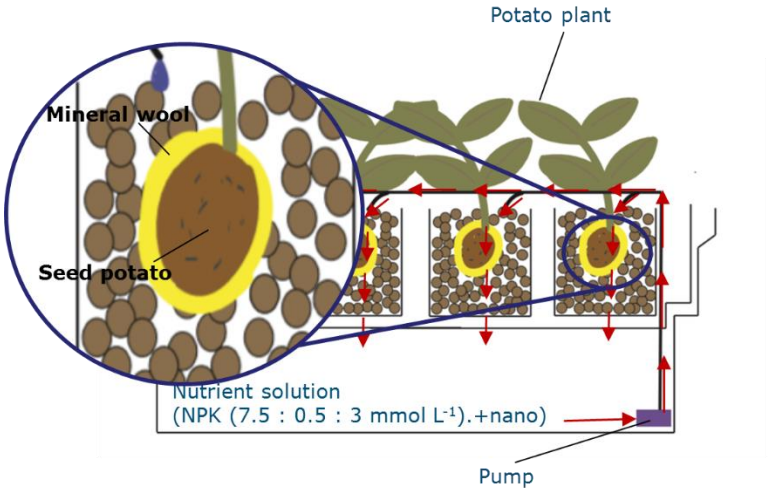


Figure 6: Schematic of the Wilma drip feed hydroponic system used in the initial trials to observe fortification of tubers using MONP

3.2. Greenhouse and polytunnel propagation (Sax2015, Sax2016)

Trials under greenhouse conditions without additional heating or light, were used to observe the influence of additional nutrients present in the compost, fluctuations in light levels, temperature and photoperiod changes, upon the MONP up take by the plants. Greenhouse trials conducted at Clifton campus and poly-tunnel trials conducted at Brackenhurst campus, followed the same strategy: three chitted seed tubers, planted in an equal lateral triangle 20 cm apart. The plants were cultivated in 40 L sacks (purchased for LBS Horticulture), planting a third of the way down as recommended. The growth medium was a peat-based Erin Multipurpose compost. The growing sacks were laid out 50 cm, Sax2016 and 15 cm apart, Sax2015 (accordance to field propagation) so the foliage did not impinge on the adjacent sack (figure 12).

Each potato plant was fed once a week with 1 ltr of MONP+His formulation. Watering of 1 litre three times a week with tap water per plant. Latter trials have used a base NPK fertiliser (Chempak, 6:5:7 + 4MgO) to simulate the response the addition MONP would have on propagation of the plant and the feasibility of introducing the MONP into a commercial feed. Table 4 summaries Sax2015 and Sax2016 trials.

Trial	*Application*	*Concentration of MONP (mg/L)*	*Chempak applied*	*Number of tubers*
Sax2015	Control	0	Y	21
	CaNP+His	12	Y	9
	CaNP+His	36	Y	9
	CaFeNP+His	Fe:12 Ca:24	Y	9
	FeNP+His	8	Y	21
	FeNP+His	12	Y	21
	FeNP+His	16	Y	21
	ZnNP+His	8	Y	9
	ZnNP+His	16	Y	9
Sax2016	Control (water only)	0	N	18
	Control with Chempak	0	Y	18
	CaNP+His	32	Y	18
	CaNP+His	64	Y	18
	FeNP+His	16	Y	18
	FeNP+His	32	Y	18
	His.	16	Y	18
	His.	32	Y	18
	His.	64	Y	18

Table 2: MONP solutions tested in trials using the potato cultivar 'Saxon' for trials Sax2015 (conducted under unheated greenhouse conditions at Clifton Campus) and Sax2016 (conducted in poly-tunnel, Brackenhurst Campus). All applications, including control, had an application of Chempak, apart from control (water only) Sax2016 where no additional feritlers were applied.

Figure 7: Sax2016 trial under poly -tunnel conditions: Brackenhurst, Southwell, Nottinghamshire.

3.3. **Field**

Application of FeNP+His differed from the trials previously conducted to replicate commercial fertiliser applications. A commercial application was used in all four trials (table 4) and laid out as in figure 13. The control cohort was treated without any additional FeNP+His (T1).

	Application	*Weeks since planting (planting = week 0)*
T1	Control - no iron	N/A
T2	Drench	0
T3	Drench + 1 foliar	0 + 7
T4	Drench + 2 foliar	0 + 7 + 11

Table 3: Details of the application of FeNP+His. using Mozart cultivar.

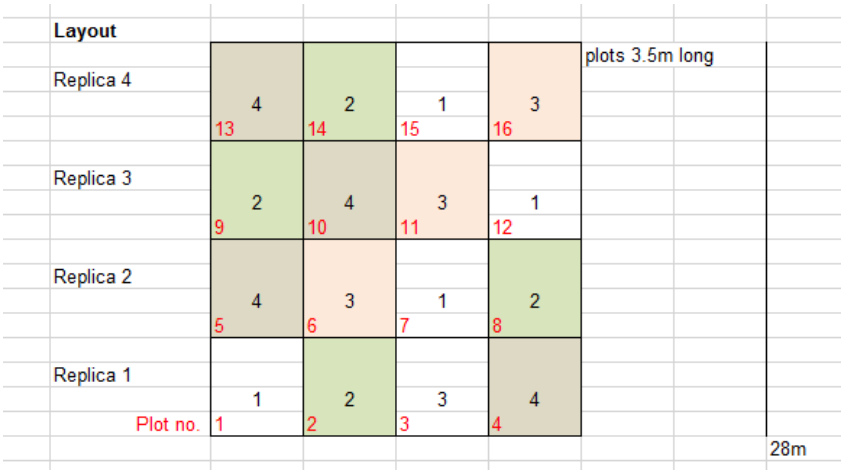


Figure 8: Field trial layout, 2015 in collaboration with Branston Ltd.

A soil drench application at planting with FeNP+His 20 mg / L was applied to the other three treatments. T1 treatment had only the drench application at time of planting. The other treatments included an addition foliar application after four weeks, T3 and T4 had a second application after seven weeks after planting. A replica trial (Field rep2016) was carried out using T1, T2 and T3 applications and conducted in a poly tunnel to monitor effects on shoot height and number along with a comparison of yield, DM% and chlorophyll levels. Treatment one consisted of once drench application FeNP+His 30 mg/ L, with a second application in treatment two of the same concentration at tuber initiation.

The cultivar 'Swift' was used to replicate the field trial conducted in collaboration with Branston Limited. All applications commenced with a drench application at planting, FeNP+His., 50 mg/L, (coinciding with Field2016) with foliar application at week 5, FeNP+His., 50 mg/L (Field2015). In order to protect the surrounding plants from contamination the plants where protected by two layers (1 mm thick) of plastic sheeting screen. A Hozelock 4122 Spraymist 1.25L, purchased from B&Q, was used to apply 1L FeNP+His., 50 mg/L, to each bag consisting of three potato plants. The chitted tubers were cultivated as in previous trials at the Brackenhurst Campus, consisting of 15 tubers per treatment. At week 13, the tubers were harvested. This trial was used to observe the growth rate response to the Fe applications.

3.4. **Data collection**

Foliage growth was measured via shoot height (apart from plants propagation in the field trials), from two weeks after planting (w.a.p.). Flowering was noted as a secondary effect

of MONP application. At the point of harvest the fresh weights (2 d.p.) and numbers for trials conducted in Clifton and Brackenhurst were obtained and divided into two categories, >30mm and <30 mm.

Branston Ltd provided the number and average harvested weights for Field2015, further divided into sizes <20, 20-40, 41-65 and <65mm. No yield data was collected from hydroponic propagation due to the restrictions of the pots used.

For trials Sax2015, Sax2016, Feload2016 and Fieldrep2016, the DM% of Dry mass of a similar sized tubers (100 mm length, 30 g) were selected (n >10 per application). Using this regulatory system enabled tubers of similar age / growth stage to be analysed. Branston supplied the tubers from Field2015 (n=8 per treatment) and Field2016 (n=10 per treatment).

Each tuber was washed with distilled water twice, patted dry and left to dry at room temperature for 30 mins. A central core of a potato was taken (diameter 15mm) using a cork border from the bud end to the stem end (figure 14) and immediately weighed. The sample was then place in dehydrator at 65^{o}C, and reweighed until a consistent dry weight was obtained (10-15 hours).

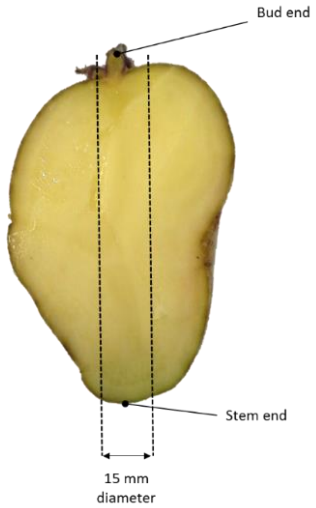


Figure 9: Sampling a potato tuber for DM%

Soil particles could interfere with mineral content analysis therefore contamination was avoided by roughly washing the tubers in deionised water twice, patted dry and left to dry at room temperature for 30 mins. Using a cork border (diameter 15mm), a core sample taken from the bud end to the stem end (figure 13) used in DM% was use to give an over view of the mineral content of the whole tuber. The constituent parts of the potato is identified in figure 15. Two horizontal core same were taken and divided into three parts central core of a potato was taken (figure 16). Each sample was dried as previously described for DM %. All samples where ground to a fine powder using a Tefal GT203840 Coffee Grinder.

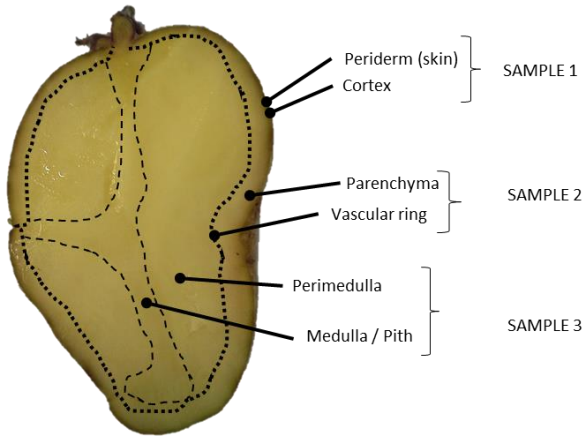


Figure 10: Constituent parts of the potato [32]

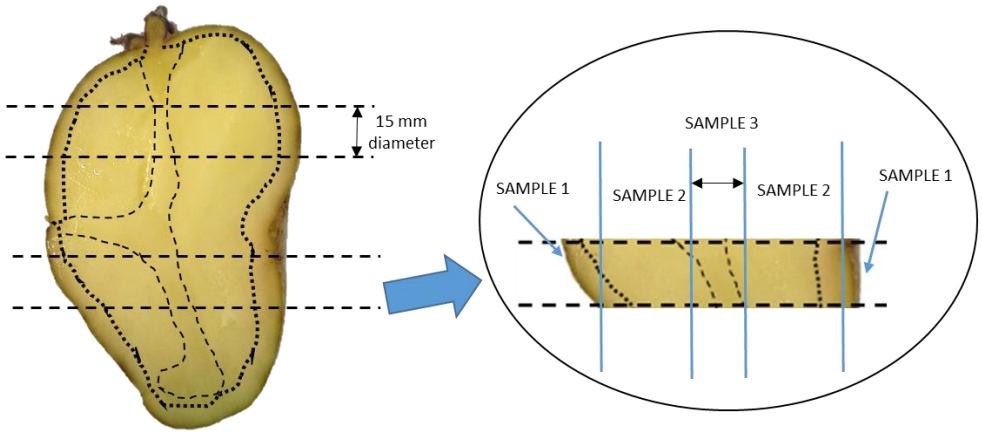


Figure 11: Tuber samples taken for ICP analysis.

Digestion of dried potato tubers was carried out using ETHOS UP High Performance Microwave Digester System using the pre-set methodology '*Dried plant material'*. Chemicals used for the digestions were purchased from Sigma-Aldrich (hydrogen peroxide) and Thermo Fisher (Nitric acid, 36 %, analytical grade). The mineral content of the samples was obtained by Perkin Elma ICP-OES Opmtima 2100 DV, using calibrated using a serially diluted standards purchased from Fluker. The fully digested material solution was diluted to 20% for ICP analysis, to avoid nitric acid corroding the feed lines. The mineral content was then calculated to 1g of dried sample. The data was statistically analysed using ANOVA single factor, SD <p=0.05.

Soil samples were dried in a dehydrator at 65ºC for 20 hours and ground to a fine powder using a Tefal GT203840 Coffee Grinder. Digestion of compost and soil samples were carried out using ETHOSUP High Performance Microwave Digester System using the pre-set methodology '*BSC 300 (soil).* All organic material was fully digested using nitric acid, 36 %, 10 mL apart from sand particulates. The fully digested material solution was diluted to 20% for ICP-OES analysis.

3.5. Observation of Fe uptake using radioactive isotope ^{59}Fe.

To directly compare the uptake of Fe from FeNP+His. and commonly used iron delivery method of Fe-EDTA, both iron compounds were synthesised using the radioisotope ^{59}Fe, 1 mCi, purchased from Perkin Emler. The isotope allows tracking of the iron through the plant as well as quantity of iron utilised through the plant. Thirdly, the retention of iron in the soil can be observed. Chemicals for Fe-EDTA synthesis were purchases from Sigma Aldrich and the precursor chemicals for the FeNP+His. synthesis was purchased as for pervious method.

The synthesis of this trial was adapted from Lauret *et al*. (2008) [56] were ^{59}Fe solution (10 mL) was added to a solution of iron (III) chloride (0.1 mol^{-1}, 30 mL) and iron (II) chloride (0.05 mol^{-1}, 30 mL), a 2:1 ration by molarity. This mix was added by continuous drip via a pressure equalising funnel, into sodium hydroxide (3 mol^{-3}, 60 mL). The sodium hydroxide was heated to 60^{o}C with continuous stirring at 500 rpm in a 250 mL a round bottom flask for 1 hour. The black nanoparticles were filtered through a grade 2 glass sintered funnel via vacuum filtration and washed with deionised water (3 x 50 mL) then ethanol (20 mL) and left to dry over night before in fume hood being ground for further use. Equal weight of histidine monochloride to iron oxide nanoparticle was ground using a pestle and mortar. The ^{59}FeNP+His., (3.30 g) was suspended into distilled water (1000 mL) making a stock solution. The stock solution (66.6 mL) was diluted to into distilled water (433.4 mL) before application to the plants.

The synthesis of Fe-EDTA [62] involved the preparation of two precursor solutions; Solution A: Disodium EDTA (1.9g) into a solution of sodium hydroxide (1 mol^{-1}, 5 mL); Solution B: Iron (III) chloride hexahydrate (1.25 g) into distilled water (2.5 mL). Solution A was added to solution B with continual stirring and heated to 60 ^{O}C until a yellow precipitate formed. The precipitated was obtained by filtration and washed with ice cold water (2 x 50 m) and once with ethanol (20 mL). No coating method was required as Fe-EDTA is soluble. Fe-EDTA (4.64 g) into distilled water (1000 mL). The stock solution (66.6 mL) was diluted with distilled water (433.4 mL) before application to plants.
The treatment (500 mL) was added once a week to the potato plants (planted in multipurpose compost as in previous trials) and watered every other day with tap water for six weeks. Three replicates of each application were cultivated. Samples of the compost, tuber and stem (lower, mid and upper) were taken and analysed for gamma radiation activity using a Hidex AMG Gamma Counter.

3.6. Antibacterial properties of CaNP+His solution

Wash water (3 ltr) from potato washing was collected from Produce World, Sutton Bridge, and used as a general bacterial source. Buffered solutions of MONP+His (CaNP and CaFeNP) and His only solution (100 mg / L, 20 mL per rep) were inoculated with wash water (500 µL) and incubated for 2 hours at room temperature. The solutions where diluted 10^6 and spread onto nutrient agar plates with further incubation (24 hours at 17 ^{o}C). The colonies were then counted and converted into CFU / ml.

3.6.1. Inoculation of potatoes (variety Maris piper) with Pectobacteria pre-treated with MONP+His soak

Eight Maris piper potatoes were washed in a commercial washer (figure 17) and placed in the wash water at ambient temperature for two hours to inoculate the potatoes with Pectobacteria. The potatoes were sampled by skin swabs and peel. A 25 mm^2 area of

skin was swabbed in three areas and a peel sample was taken, figure 18. Peel samples were obtained with the use of a food grater, from the radius around the tuber (diameter of 10 mm) from apical to bud end until 2.5 g of skin was obtained. The peeling was homogenised with sterilised water (5 mL) and filtered gravitationally through a grade 1 filter paper.

Figure 12: Maris Piper tubers prepared for the trial, Sutton Bridge Crop Storage Research Centre

Figure 13: Skin swabbing process of tubers

The skin swabs and peel samples were diluted (skin, 100 x and peelings 1000 x) through serial dilution and with sterilised distilled water. Samples were spread onto nutrient agar plate with two replicates of each sample. The plates were incubated at 17^{o}C for 24 hrs. The Pectobacteria colonies leave wells in the agar which were counted to obtain a concentration figure of the bacteria and quoted as CFU in mL.

3.6.2. Inoculation of potatoes with Pectobacteria (PCA).

Initial testing using variety Maris Piper used three tubers per test. Two treatments were applied, calcium oxide (CaNP), 200 mg/ L and calcium ferrite (CaFeNP), Ca concentration of 200 mg / L both coated in amino acid histidine to aid dissolution in deionised water (2000 cm^3 di. Water per treatment).

3.6.3. Test 1: No pre-treatment

Nine tubers were washed in a commercial washer until clean (approx. 5 mins) then divided into the following treatments: Control (no treatment), CaNP+His and CaFeNP+His. These

were left to soak at ambient temperature for 24 hours, figure 19. The tubers were removed from the solution then placed into PCA solution (10^6 concentration) for two hours at ambient temperature to induce inoculation via lenticels s as in figure 19.

Figure 14: Tubers soaked in treatment solutions before inoculation

3.6.4. Test 2: Produce wash

Tubers (x 8) were washed in Produce Wash in a dilution of 1:200 as recommended by the manufacture. The tubers were divided into three per treatment and treated and inoculated as in test 1, see table 6.

The skin swabs and peel samples were diluted (skin, 100 x and peelings 1000 x) through serial dilution and with sterilised distilled water. Samples were spread onto nutrient agar plate with two replicates of each sample. The plates were incubated at 17ºC for 24 hrs. The pectobacteria colonies leave wells in the agar which were counted to obtain a concentration figure of the bacteria and quoted as CFU in ml.

	Conditions	*Washing*	*Treatment (24 hr soak) @ RT*		
1	Control	Water	Water	*PCA soak 2 hours @RT*	*Swab and peel samples taken onto LB plates (2 reps per sample)*
2	Water/CaNP+His.	Water	CaNP+His		
3	Water/CaFeNP+His.	Water	CaFeNP+His		
1a	PW/ no treatment	PW	Water		
2a	PW/CaNP+His.	PW	CaNP+His.		
3a	PW/CaFeNP+His.	PW	CaFeNP+His.		

Table 4: Washing application and post-wash treatment with application of CaNP+His and CaFeNP+His

4. RESULTS

4.1. Stem growth rate.

4.1.1. Hydroponic (H2014 and H2015)

From the data collected in trial H2014, figure 20 and table 7, it is observed the 8 ppm FeNP+His 8 mg / L and His. 8 mg / L has the optimal growth. Overall, the Zn+HisNP treated potatoes are significantly suppressed by the presence of ZnNP with increases of 13.73 mm and 13.18 mm, ZnNP+His 8 and 16 mg / L respectively.

	p-Value
Control against ZnNP+His 8 mg / L	1.81×10^{-9}
Control against ZnNP+His 16 mg / L	1.4×10^{-9}

Table 5: p-value of stem heights (trial H2015) at week 5 of cultivation showing significant (<p=0.05) decrease in heights using ANOVA single factor analysis.

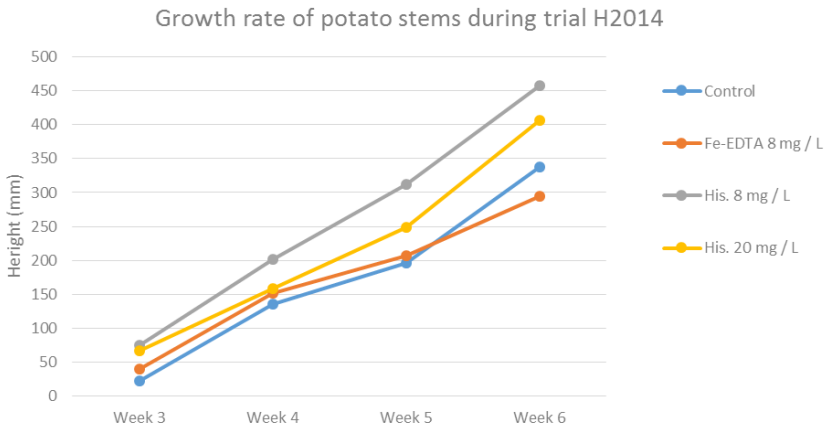


Figure 15: Growth rate of potatoes in trial H2015. Control plants increased by av. 304.22 mm, Fe-EDTA by 237. 22, His. 8 mg /L by 368.94 and His. 20 mg / L by 340.17 mm

Ca+HisNP 12 mg / L did not grow as rapidly as expected with a height increase of 31.43 mm when compared to the increase of 216.63 mm obtained by Ca+HisNP 32 mg /L (figure 21a).

The tubers treated with His 8 mg / L significantly increase in height (p = 0.000109) compared to the increase in height gained by control plants, figure 20. The height increase gained by His. 20 mg / L. although an increase over control was not significant.

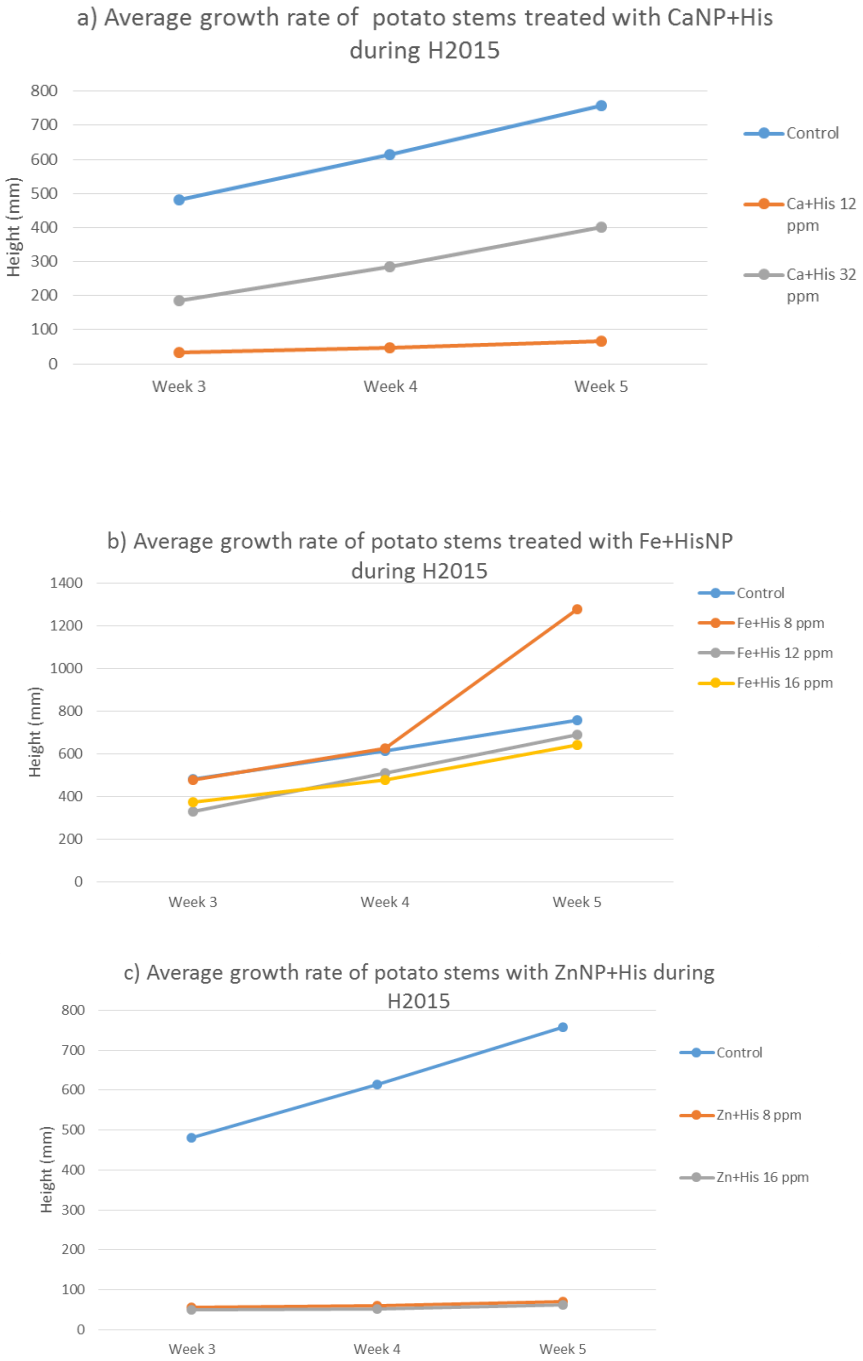


Figure 16:The growth rates of plants treated with CaNP+His (a) and ZnNP+His (c), have suppressed growth when compared to control. FeNP+His 8 mg / L (b) is the only treatment in the trial that demonstrated an increase in growth rate over control plants.

4.1.2. Sax2015 and Sax2016

Sax2015 and Sax2016 growth rate found no sig. dif. when comparing the increase in height between weeks 3 and 5 (in conjunction with H2015) in Sax2015, figure 22. A percentage increase in height over control plants can be observed for treatments FeNP+His 16 mg / L, 9.90 %, CaFeNP+His., 5.40 % and ZnNP+His. 16 mg / L (figure 22).

Potato stem heights after six weeks from planting, a 2.98 % increase over control gained by plants treated with FeNP+His. 12 mg / L and 0.26%, with plants treated with FeNP+His.

16mg / L, these increases were not found to be of significance, table 8. Plants fed with CaNP+His. 12 mg/ L gained 7.03 % increase over control height but was not found to be significant. However, CaFeNP+His and ZnNP+His 8 mg / L gained significant difference over control height stems, table 8. It was observed that between week 5 and 6 (figure 22) the control plants growth rate reduces as the plant commences the tuber filling stage (40 + days after planting). Plants treated with CaNP, FeNP and CaFeNP sustained growth rates during this period.

	Mean height (mm) at 6 weeks	*Percentage + or - in height against control*	*p value using ANOVA single factor*
Control	1198.73	N/A	N/A
FeNP+His 8 mg / L	1140.47	-4.86	0.2023
FeNP+His 12 mg / L	1234.40	2.98	0.2023
FeN+His 16 mg / L	1201.87	0.26	0.9246
CaNP+His 12 mg / L	1283.00	7.03	0.0501
CaNP+His 36 mg / L	1164.70	-2.84	0.2982
Ca.FeNP+His	1318.00	9.95	0.0210
ZnNP+His 8 mg / L	1300.80	8.51	0.0201
ZnNP+His 16 mg / L	1169.50	-2.44	0.7160

Table 6: Height (mm) of potato stems, percentage of height increase or decrease when compared to control six weeks after planting. P values attained from ANOVA single factor comparing control heights and treated plant stem heights at six weeks of growth. A p value < 0.05 was deemed significantly different

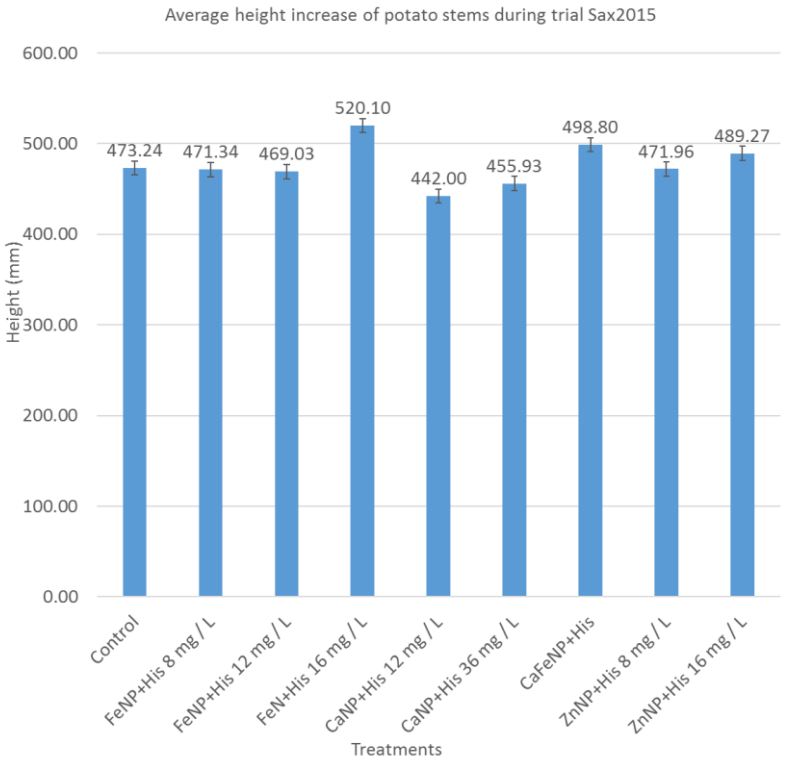


Figure 17: Average height increase of potato stems between weeks 3 to 5 after planting.

Growth rate data collected from trial Sax2016 showed sig. dif. of stem heights gained between weeks 3 to 5, figure 23. CaNP+His 32 and 64 mg / L has a significant increase in height over control, Chempak and the His. equivalent suggesting an influence in the presence of CaNP. This is supported by figure 24a where there in an increase in growth rate in stems treated with CaNP+His 32 mg /L.

His. only applications increase the growth rate of the stems as observed in figures 24 a,b,c,d, with a greater significance at lower concentrations.

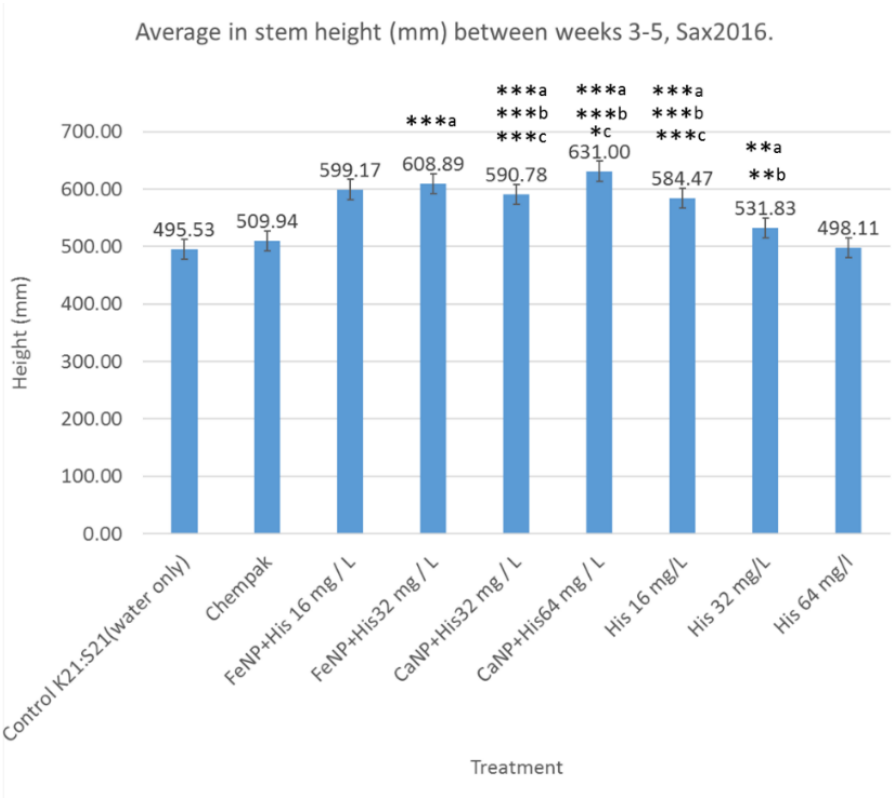


*Figure 18 Average increase in height of potato stems between weeks 3 and 5 after planting to be in conjunction with previous trials. Using ANOVA single factor statistical analysis, p-values were ranked; * p=0.05>, ** p=0.01, *** p=0.005. Letters a, against Control; b, against Chempak and c, against MONP and His. equivalent.*

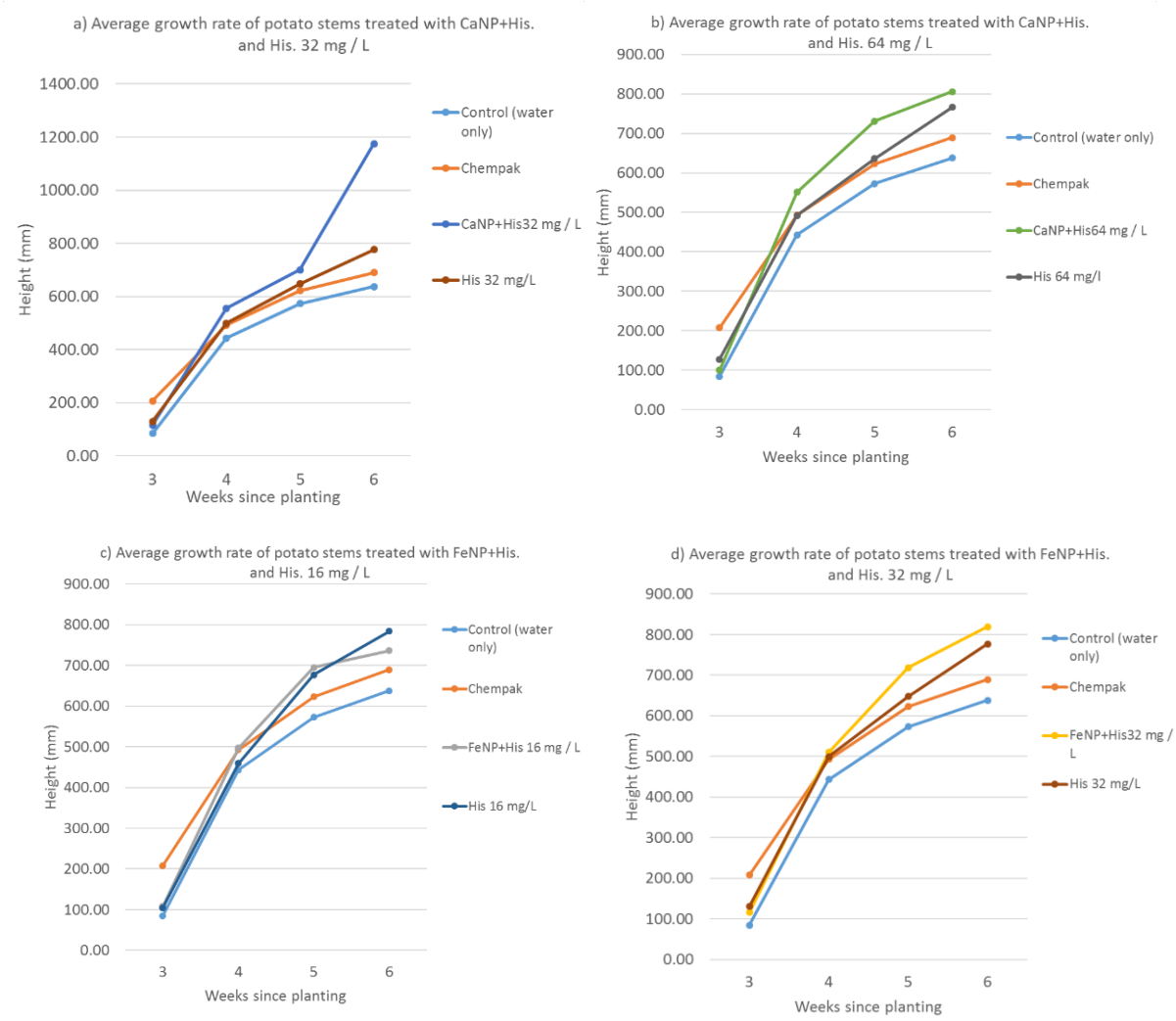


Figure 19: Average growth rates of potato stems in trial Sax2016. Control and those treated with commercial fertiliser, Chempak has a reduced height in stems than those treated with amino acid histidine or MONP's.

4.1.3. FieldRep2016

From the data collected (figure 25), the growth rate of the stem is sustained into the tuber loading phase of the potato plants growth cycle. Using ANOVA single factor there is a sig. dif., p=0.000109, between 'control' and 'Drench + 5-week app.'

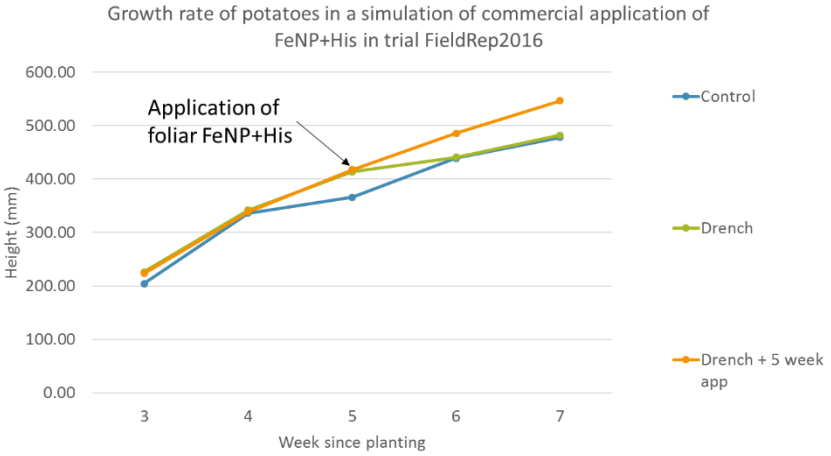


Figure 20: Average growth rates of potato stems in FieldRep2016 trial

4.2. Effect of MONP of yield

4.2.1. Sax2015 and Sax2016

When comparing percentages variations, a 10 % increase or decrease was taken to be of significance with a H_0 ; "*applications of MONP do not influence the number of tubers harvested or the physiological maturation of the tubers"*. This H_o was used when performing a Chi Squared statistical analysis, X^2.

The average number of tubers harvested per plant in Sax2015 were observed to increase over control when treated with FeNP+His 8 mg / L (10.31 %), CaNP+His 32 mg / L (25.83 %) which can be deemed as significant. FeNP+His 12 mg / L yield was not significantly less than control, however, FeNP+His 16 mg / L produced a significantly lower yield (-17.05 %) compared to control. The higher CaFe NP+His 24:12mg/L application had the opposite effect the 32 mg / L had on yield, with a significant loss of 33.59 %. Although other FeNP and CaNP treatments obtained a lower number of tubers, when comparing growth data (figure 22) FeNP+His 16 mg / L gained increased stem height, surmising there is no overall negative effect of Fe or CaNP application.

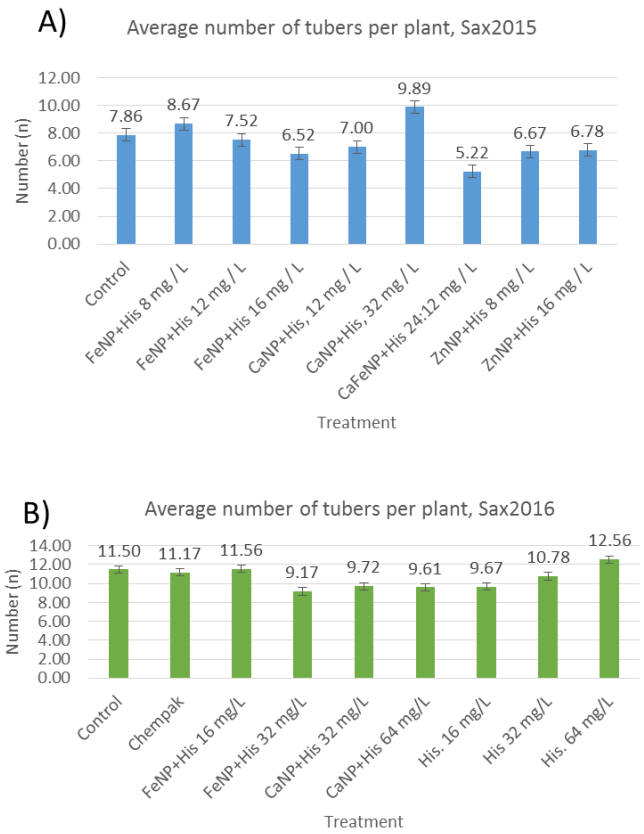


Figure 21: Average number of tubers harvested per plant. Comparing Sax2015 (A) and Sax16 (B) trials using Saxon cultivar

It was observed in figure 26A, the application of ZnNP+His and CaFe+His had a significant negative effect on the number of tubers, producing a 15.14% and 13.74 % reduction in tuber numbers from ZnNP+His 8 and 16 mg / L respectively. The application of CaFeNP+His obtained the least number of tubers with a reduction of 33.59 %.

Sax2016 trial control (no Chempak or MONP) gained a higher yield than those treated with Chempak by 2.95% concluding that the Chempak did not significantly impact on yield, figure 26B. Those treatments that gained a higher yield than Chempak where FeNP+His 16 mg / L (3.49 %) and His. 64 mg / L (12.44 %). Yield loss compared against control ranged from 22.19 to 6.36 % and loss of 17.91 to 3.49 % against Chempak. Sax2015 losses had a more significant and wider range of percentage loss of 33.59 to 4.33 %.

In figure 27B, it was observed a lower variance of ± 3.39 (n) was achieved in Sax2016, compared to Sax2015 ± 4.64 (n). Comparing FeNP+His. 16 mg / L application yields between Sax2015 and Sax2016 are inconsistent leading to question the influence of viability of seed potato and environmental conditions. Variations of this kind are due to environmental and genetic variation within the cultivar [32,63] as the strata, time of year and treatment application was identical in both trials. Sax2015 were subjected to higher temperatures, due to the nature of the greenhouse conditions were as the Sax2016 plants were in a well ventilated poly-tunnel Increasing the sample number, repetitions and a focus on temperature and light fluctuations would establish the impact on such influences.

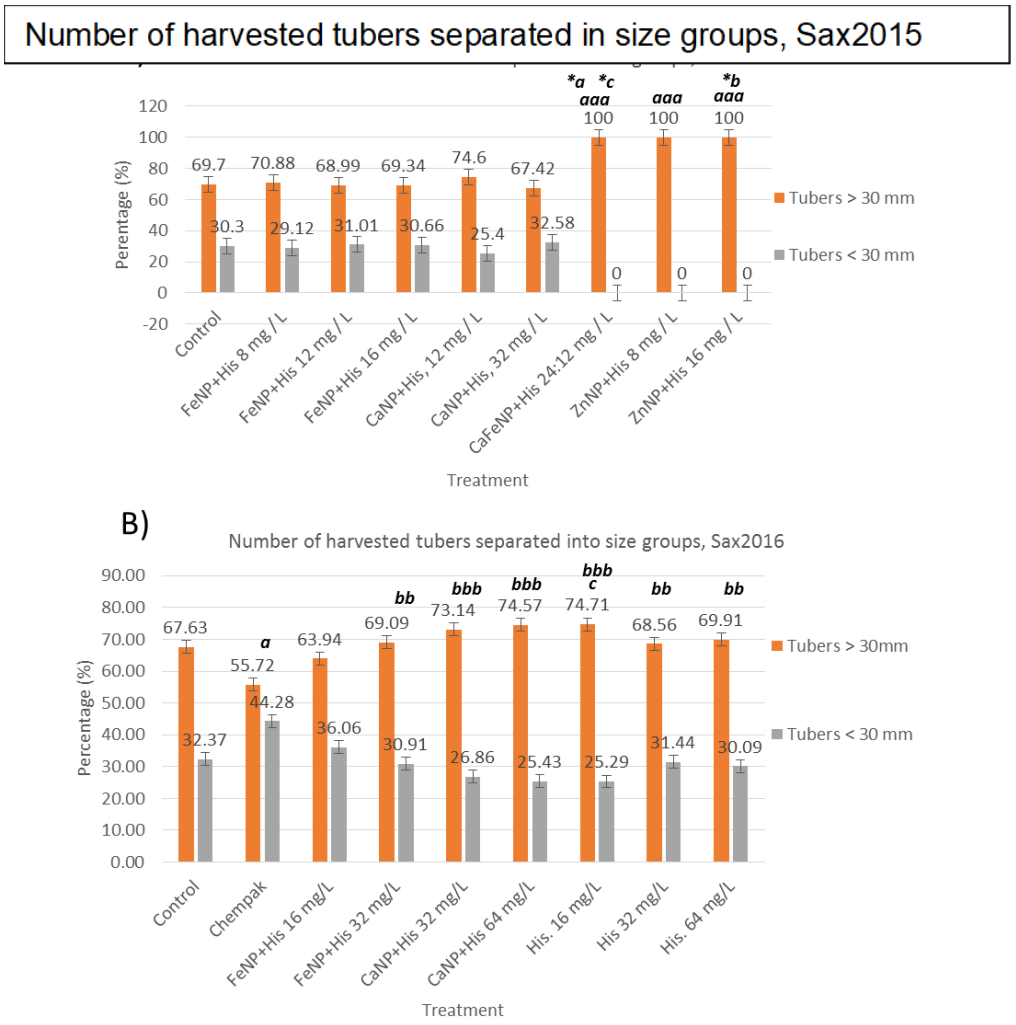


*Figure 22:Segregation of tubers into commercial acceptable size (> 30 mm) and < 30 mm. Sax2015 (A) indicates sig.dif. analysed via Chi Squared and ranked * p=0.05>, ** p=0.01, *** p=0.005. 'a' indicates a sig. dif. Between control and treatment; '*a', FeNP+His 12 mg / L against CaFeNP+His; '*b', FeNP+His 16 mg / L against ZnNP+His 16 mg / L and '*c' CaNP+His 12 mg / L against CaFeNP+His. Saxon2016 (B) using the ranking system as in A, 'a' indicated sif. dif against control, 'b' against Chempak and 'c' against MONP equivalent.*

The H_o for the percentages of > 30 and < 30 mm of harvested tubers were tested using X^2 with < p = 0.05. A sig. dif. between control and His. applications in trial Sax2015. Further sig. dif. was found in Sax2015, figure 27A, between FeNP+His 12 mg /L and CaFeNP+His; FeNP+His. and ZnNP+His 16 mg / L; CaNP+His 12 mg / L and CaFeNP+His. thus rejecting the H_o and recognising the treatments have an impact on the size of tubers harvested. The analysis was also carried out for data collated from the harvest of Sax2016, were sig. dif. against Chempak was found between, control and all other treatments apart from FeNP+His 16 mg / L as observed in figure 27A. His. 16 mg / L significantly increased the proportion of > 30 mm tubers when compared to the MONP equivalent application of FeNP+His. 16 mg/ L.

Comparing harvested data statistically via ANOVA single factor analysis, from trial Sax2015, a significant increase between control plants overall average weight (OAW) in grams, and treatments, CaNP+His 12 mg / L ($p = 0.01$), CaFeNP+His ($p = 1.43 \times 10^{-4}$), ZnNP+His. 8 mg / L ($p = 3.21 \times 10^{-5}$) was found.

No sig. dif. was found in the data collected at the time of harvest between control harvested weights of > 30 mm. It was observed that CaNP+His. 32 mg / L and FeNP+His 8 mg / L produced 25.86 and 10.30 % more tubers per control plant respectively.
The tubers harvested measuring under the commercial acceptable level of 30 mm, had a significant difference in weight against control were FeNP+His 8, 12 and 16 mg /L. Treatments CaFeNP+His and both ZnNP+His did not produce any sub 30 mm tubers. Due to the harvest occurring at 14 weeks, approximately 20 days short of commercial harvest, the occurrence of <30 mm tubers would be expected as these tubers would be used as salad potatoes. The absence of these bud tubers (sub 30 mm) indicated the plant has halted tuber initiation early into the growth cycle. The presence of ZnNP or CaFeNP did not supress the vegetable development of the treated plants, nor did individual application of FeNP and CaNP supress development as previous discussed. This anomaly required further investigation in the form of repetition of the trial on a larger scale and investigation in to possible suppression of signalling pathways involved in tuber formation.

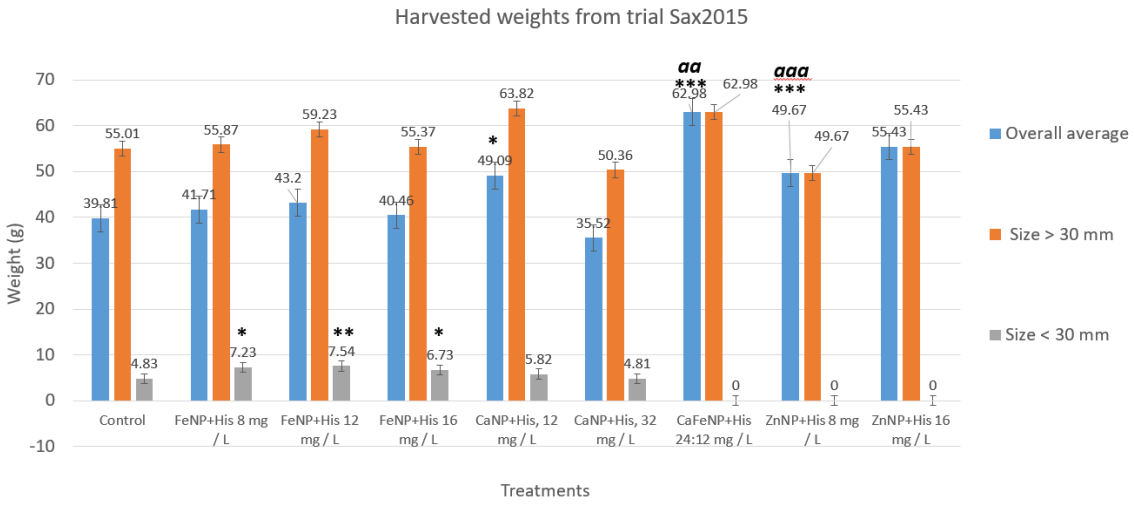


Figure 23: Comparison of harvest weights of Saxon tubers from trial Sax2015. Using ANOVA single factor statistical analysis; '' indicates a significant difference between treatment and control; 'a' indicates a significant difference between MONP of equivalent concentration*

Data collated form the trial Sax2016 display a repetition of no sig. dif. (< p = 0.05) found between control / Chempak treatments and MONP's repeated in Sax2016, for OAW of the tubers, figures 27 and 28. Treatment CaNP+His 32 mg / L displayed a 15.68 % weight increase when compared to Chempak, and a 15.60% increase against control; which is contradictory to Sax2015 results of control against CaNP+ His 32 mg / L of a 10.78 % loss.

As in Sax2015, there was a sig. dif. comparing < 30 mm between control (Sax2015) and Chempak (Sax2016) against FeNP+His 16 mg / L: p = 0.0239 in Sax2015 and a higher significance of $p = 3.38 \times 10^{-6}$ in trial Sax2016, figure 29. In the Sax2015, control treated with Chempak, as was the MONP treatments in both trials; subsequently the 'Chempak' treatment in Sax2016 is the equivalent to Sax2015 'control'.

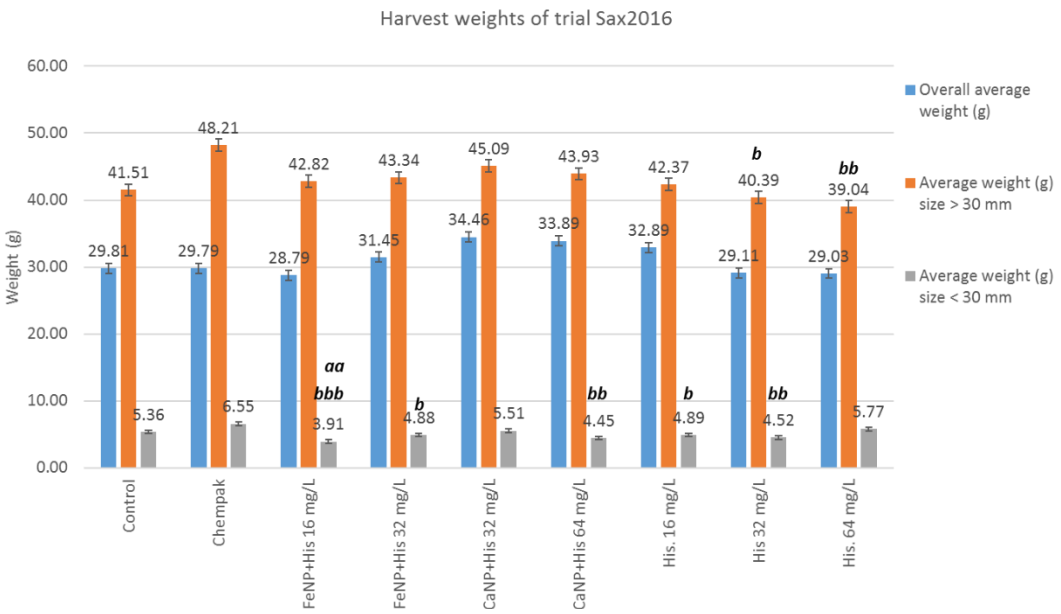


Figure 24: Comparison of harvest weights of Saxon tubers from trial Sax2016. Using ANOVA single factor statistical analysis; '*a*' indicates a significant difference between treatment and control; '***b***' indicates a significant difference between treatment and Chempak.

4.2.2. Field2015

Using a H_0 *"The application of FeNP+His. 30 mg / L would not influence the yield; number of tubers, size distribution and weight."* This was tested using AVOVA, X^2 and percentage increase/ decrease using levels of significance previously used.

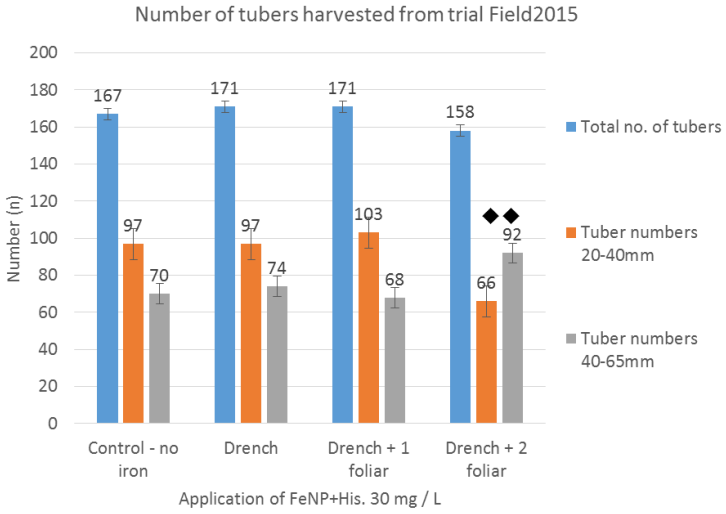


Figure 25: Number of tubers harvested from trial Field2016 in collaboration with Branston Ltd, cultivated in Lincolnshire. The "◆◆" indicates the level of sig.dif. obtained via X^2 statistical analysis between 'Control' and 'Drench + 2 foliar" application

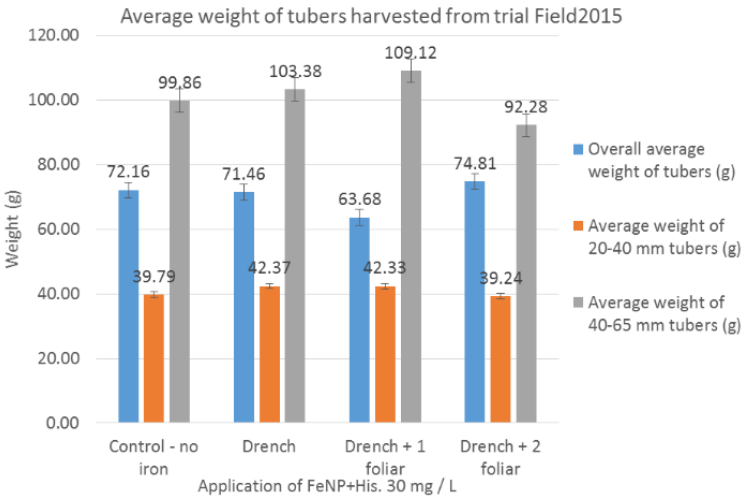


Figure 26: Harvested weights of tubers cultivated in trial Field2015 and segregated in to sizes

Comparing the total number of tubers harvested per application (figure 30), no significant difference was found using ANOVA single factor or percentage increase / decrease. Using X^2 to distinguish changes to tuber size distribution instigated by application of FeNP+His. 30 mg / L, a significant change was found between 'Control' and application 'Drench + 2 foliar'. This was supported with significant decrease in the number of tubers 20-40mm (-31.96%) and number of tubers 40-65mm (-31.43%), figures 30 and 31 concluding a second application of FeNP had a detrimental effect on yield.

4.2.3. FieldRep2016

To replicate the loading of the FeNP+His. used in the Field2015 trial, a concentration of 50 mg / L was applied at planting as a 'drench' for both 'L1' and 'L2'. A second foliar application was applied at 8 w.a.p for 'L2' application, sooner than in the field trials as a rapid cultivar Swift was used for trial 'Field rep 2016', therefore shortening the growth period and bringing forward the midway foliar application as seen in Field2015. No addition fertiliser was used for control. Due to unforeseen circumstances the trial was harvested five weeks earlier than planned, therefore an increased number of small tubers (< 30 mm) than usual were harvested. For this reason, figure 32 presents the average number of tubers per plant without size segregation.

Using the H_0^1; The application of FeNP+His. 50 mg / L had no effect on the number of tubers harvested" and a second null hypothesis H_o^2; "*the application of FeNP+His. 50 mg / L had no effect on the tuber size distribution*". Figure 32 represents the average number of tubers harvested at 10 w.a.p. L1, drench at planting only, produced 70.18 % more tubers than the control plants with L2, drench and a second foliar application at 8 w.a.p, producing 30.83 %. It can be said the application of FeNP+His. 50 mg / L increased the number of tubers produced thus H_o^1 can be rejected as both 'L1' and 'L2' > 10%.

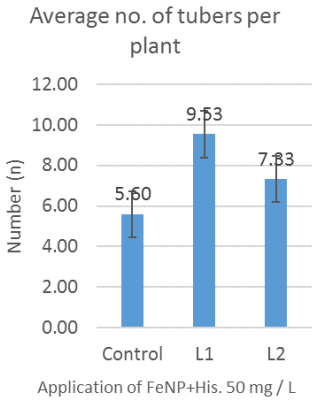


Figure 27: Average number of tubers harvested, Field rep 2016

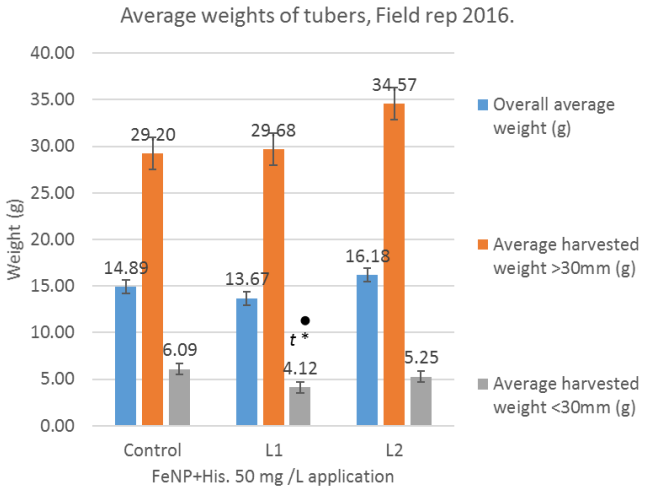


Figure 28: Harvested weights of tubers from 'Field rep 2016. 't' represents sig. dif. between control and L1; sig. dif between L1 and L2.*

No sig.dif was found between overall weights, however a significant decrease in tubers < 30 mm can be observed in figure 33, between 'Control' and 'L1' (p = 0.0250, *t*-Test one way), 'L1' and 'L2' (p=0.0282, ANOVA single factor). A pattern of increased Fe concentration producing less < 30mm can also in observed in trials Sax2015 and Sax2016, figures 30,31 and 33, where the increased exposure of Fe (loading 1) decrease the <30mm tubers. The < 30mm harvested in 'L2' are not credible in the accounting for effect of the second foliar application, as only 2 weeks had preceded since application, not allowing time for the tuberisation / loading response to be observed.

4.3. Dry mass percentage (DM%)

4.3.1. Saxon trials: Sax2015 and Sax2016

The DM % results from Sax2015 trial, presented a sig.dif. decrease when compared to control against treatments FeNP+His. 12 mg / L ($p = 1.5 \times 10^{-3}$), CaFeNP+His. ($p = 1.26 \times 10^{-2}$) and both ZnNP+His applications (8 mg / L, $p = 1.28 \times 10^{-2}$; 16 mg / L, p = 0.05)

when using ANOVA single factor analysis. It was also noted that the standard deviations for DM% was higher than control in treatments FeNP+His. 8 and 12 mg / L and CaNP+His. 32 mg / L, table 9. Treatments that have similar DM % to control (36.67% ± 3.33) are FeNP+His. 16 mg / L (33.03 % ± 2.32) and CaNP+His. 32 mg / L (36.29 % ± 3.60). FeNP+His. 16 mg / L treatment gain a similar yield to control 6.52 tubers per plant to 7.86, whereas the CaNP+His. 32 mg / L gained a significant 25.83 %, concluding that this treatment would be preferable for fry processing and long-term storage [63].

The industry requires a reliable high DM % ,20-25% [40] in order for an optimise production and continuity of product quality. Tubers below DM = 20 % increase in bruising during harvest, disintegrate during cooking and take more time and energy to process resulting in darker product which is less desirable by the consumer. Tuber flesh with a good DM, absorb less oil when frying with a higher chip yield [64], desirable texture and flavour. Both trials produced tubers above 25 % as the tubers did not undergo prolonged storage, thus retaining matter that would normally degrade.

Treatments Sax2015	*Average % DM (± SD)*
Control (with Chempak)	36.67 ± 3.33
FeNP+His 8 mg / L	35.69 ± 3.35
FeNP+His 12 mg / L	32.67 ± 4.24 **
FeNP+His 16 mg / L	35.03 ± 2.32
CaNP+His 12 mg / L	35.24 ± 2.45
CaNP+His 32 mg / L	36.29 ± 3.60
CaFeNP+His (24:12)	33.44 ± 2.14 *
ZnNP+His 8 mg / L	33.39 ± 2.85 *
ZnNP+His 16 mg / L	34.17 ± 2.01 *

*Table 7: Dry mass of tubers (n = 10) harvested from Sax2015, ± SD. Significant difference found using single factor ANOVA are indicated and ranked by **

Treatments Sax2016	*Average % DM (± SD)*
Control	39.59 ± 3.87
Chempak	38.08 ± 3.19
FeNP+His 16 mg/L	38.95 ± 2.53
FeNP+His 32 mg/L	37.87 ± 2.79
CaNP+His 32 mg/L	35.61 ± 3.08 *aaa b*
CaNP+His 64 mg/L	39.67 ± 2.64
His 16 mg/L	36.69 ± 4.04 *a*
His 32 mg/L	37.72 ± 2.71 *aaa*
His 64 mg/L	49.92 ± 5.45 *b*

Table 8:Dry mass of tubers (n = 10) harvested from Sax2016, ± SD. Significant differences using ANOVA are indicated by 'a' against control and 'b' against Chempak. Differences are ranked as previously described.

The DM % data collected form Sax2015 ranged from 32.67 % (FeNP+His. 12 mg / L) to 36.67% (Control), 4% difference; where as Sax2016 ranges from 35.61 % (CaNP+His. 32 mg / L) to 49.92 % (His. 64 mg / L), a 14.31 % difference, table 10.
Significant differences were found, but as significant decreases in all treatments apart from His. 64 mg / L (table 10). Chempak treatment obtained a slightly lower DM% than control but this could be due to a number of factors. DM % can vary between tubers from the same plant, between cultivar, storage conditions, location, mineral composition of strata and tuber [32,63]. Locational changes affect DM, yield and growth rates are concerned with soil, large altitude range, weather influenced i.e. temperature and rainfall, strata composition and mineral availability. To eliminate these influences, the same cultivar was used from the same seed potato producer, brand of compost, time of year, as well as containers with the application and watering regime. The DM samples were taken from tubers of similar size, to ensure similar chronological age and taken within 72 hours of harvest thus reducing storage influence. Location changes between trials were minimal as Clifton greenhouse coordinate are; 52.90594N, 1.19332W, altitude 53 m; Brackenhurst poly-tunnel 53.06321N. 0.96585W, altitude 72 m.

4.3.2. Field2015 and Field2016

The DM % of tubers exponentially increase after tuber initiation occurring 30 – 40 days after planting [32] at a liner increase until a foliage senescence [36] at approximately 90-120 days [32]. This rate is influenced by genetic and environmental variations [32,65]. Comparing data with that published by Kolbe and Stephan-Beckmann, 1997 [65], the DM % loss at harvest was comparable. When comparing the two data sets, Kolbe [36] use d.a.e (days after emergence) which commences on the day the seed tubers are taken out of storage and allowed to chit. A period of 14 days is allocated until the seed tuber is planted, therefore in order to compare two data sets, 2 weeks is added to the data collected from trial Field2015 where the period is measured in weeks after planting (w.a.p.).

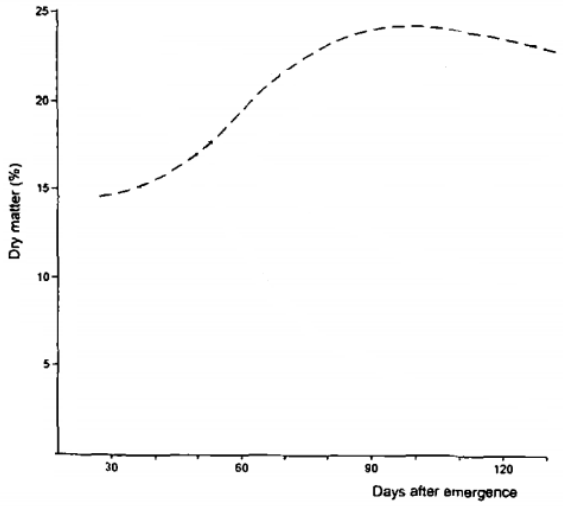


Figure 29: Development of dry matter in potato tuber over time. Adapted from Kolbe and Stephan-Beckmann, (1997) [36]

Using the following null hypothesis H_o[1] *"The application of FeNP+His. 30 mg / L does not affect the DM % of the tuber at harvest"*; and an alternative hypothesis H_1 *"The application of FeNP+His. affect the DM % loss."* which will be signified by the lack of sig.dif. DM % decrease.

Published data collated over two seasons [36] demonstrating the DM % variations throughout the tubers growth stage (figure 34) with a decreased of 6.25 % from optimal dry weight (378 g, 24 %) at 105 days after emergence (17 w.a.p.) until harvest at 135 days after emergence (21 w.a.p), 356 g (22.5 % DM).
A number of tubers were collected at 12 w.a.p, n = 20, during trial Field2015. Using ANOVA single factor statistical analysis, there was a very high sig. dif. (<p=0.001) of DM % loss between DM % 12 and 22 w.a.p for 'Control', 'Drench' and 'Drench + 2 foliar' (figure 35). The application 'Drench + 1 foliar' attained a lower DM% loss of 3.45 %. All applications of FeNP+His. reduced the DM % loss compared to 'Control'. Therefore, H_1 is accepted for the application 'Drench + 1 foliar' due to the lack of significant decrease in DM %.

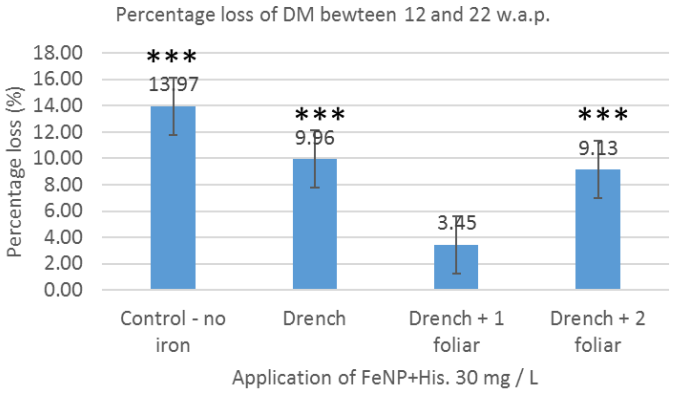
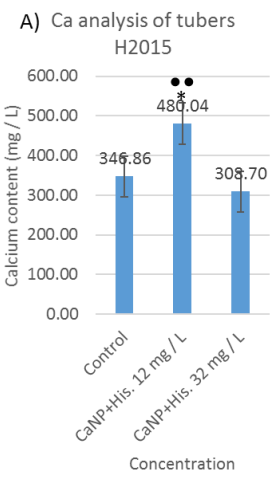


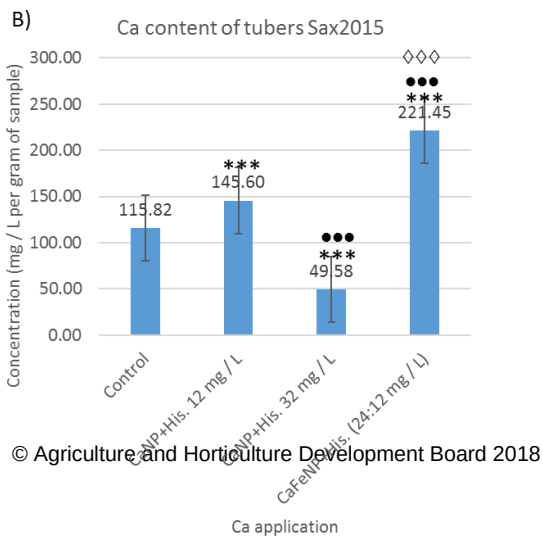
Figure 30:Percentage loss of DM % between 12 and 22 w.a.p.

4.4. The effect of MONP on mineral content of tubers

4.4.1. Ca fortification: H2015, Sax2015, and Sax2016.

Under hydroponic conditions, CaNP+His. 12 mg / L obtained a sig. dif. increase in Ca content compared to control and the higher Ca application of 32 mg / L, which tubers contained similar amounts of Ca to the control tubers (figure 36A) ANOVA single factor analysis. Using a null hypothesis, H_o; "*application of CaNP does not influence the content of Ca in potato tubers",* is rejected for applications of CaNP+His. 32 mg / L in H2015 and Sax2015. The H_o is again rejected for the application if CaFeNP, as the Ca content of the tuber is significantly higher than other CaNP applications and control. Comparing Ca fortification of concentrations 12 and 32 mg / L, the average concentration of 221.45 mg / L from the tubers fortified with calcium ferrite suggested the presence of Fe, increases Ca uptake resulting in fortification of the whole tuber. It has been published that the uptake of Ca^{2+} is not only regulated by the available Ca^{2+} in the rhizosphere but also by the presence of other ions, including Fe [32,66].





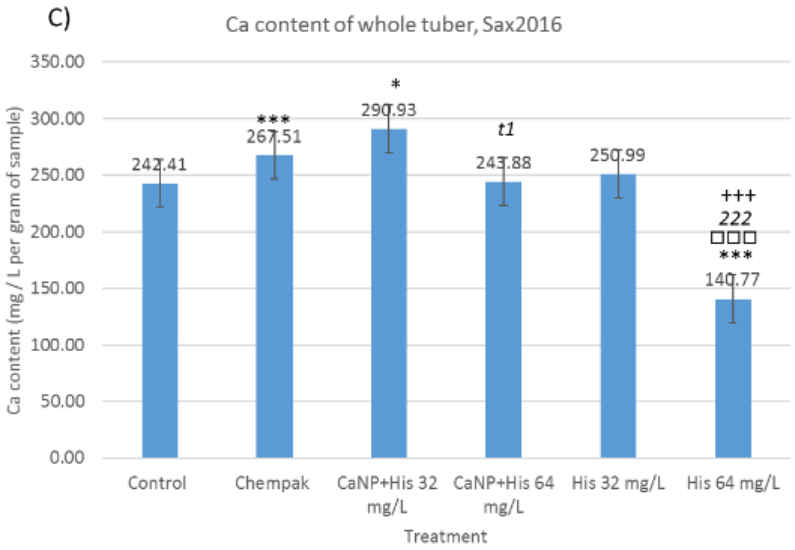


*Figure 31: Ca content of whole tuber A) H2015; influence of MONP in a hydroponic system on the mineral content of tubers. Sig.dif. found between Ca applications = •. B) Sax2015; calcium content of tubers propagated in greenhouse conditions in multipurpose compost. Sig. dif. indicated by the following: * = against control, • = against 12 mg / L, ◊ = against 32 mg / L. C) Sax2016; Ca of tubers cultivated under poly-tunnel conditions in multipurpose compost. Sig. dif. indicted by: * = against control, □ = against Chempak, t1 = CaNP+His. 32 mg / L, 2 = CaNP+His. 64 mg / L, + = His. 32 mg / L. Sig. dif. obtained via ANOVA, where indicated by 't' the sig. dif. obtained via t-Test two sample*

Sax2015 and H2015 results (figure 36 A and B) suggested the optimal Ca fortification feed would be 12 mg / L in the absence of Fe. This was not repeated in Sax2016 (figure 36C) as the results display a significant increase between control and 'CaNP+His. 32 mg / L', p = 0.0202, and a significant decrease in Ca content between Ca concentrations 12 and 32 mg / L when analysed using t-Test, p = 0.0374. There was no significant increase or decrease in the Ca content when compared to 'control' or 'Chempak', therefore accepting H_o for this application. The influence of histidine was investigated and found to achieve Ca concentrations that were not significantly different (His. 32 mg/ L) or a significant loss, indicating the presence of calcium oxide nanoparticles have successfully increased the content of Ca in the potato tubers.

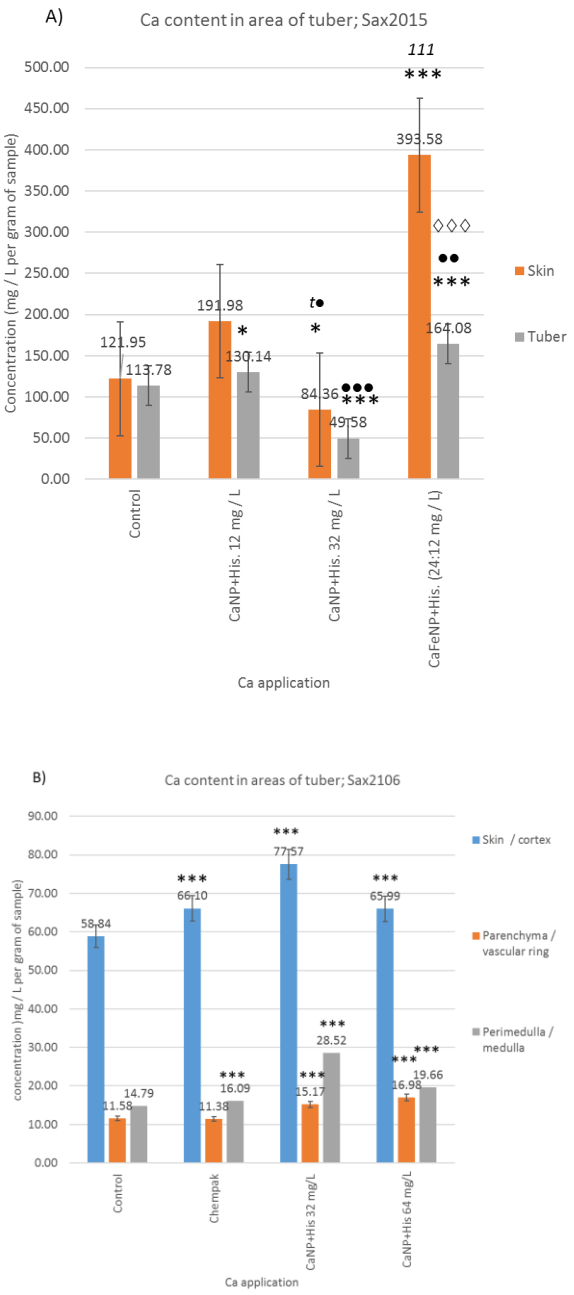
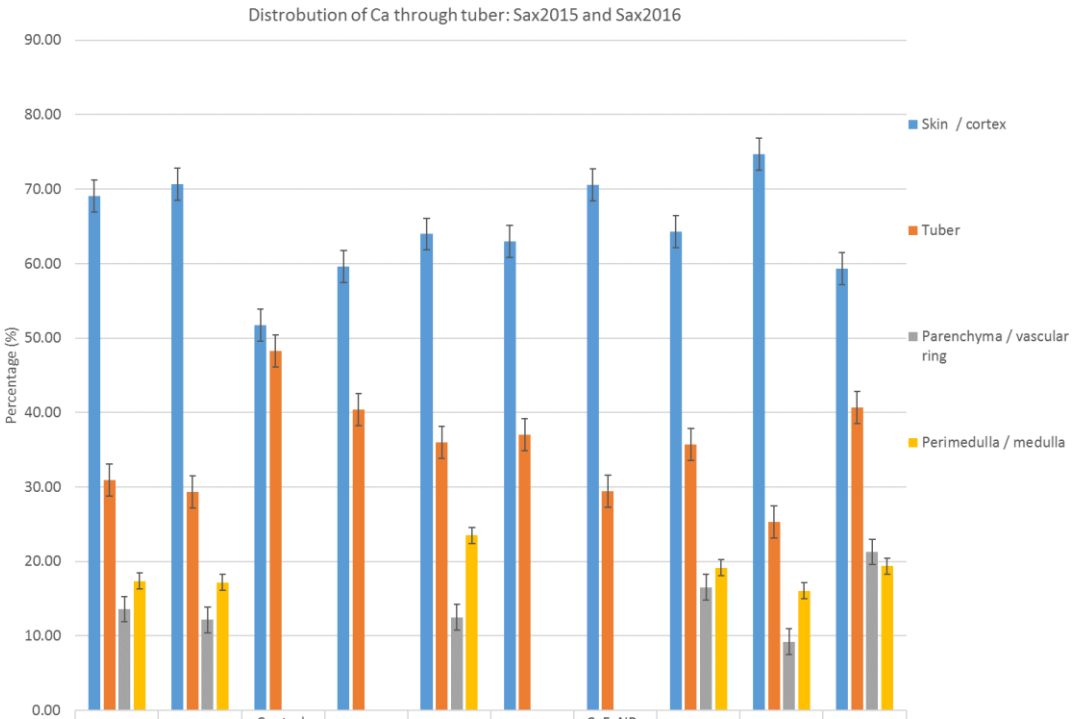


*Figure 32: Ca content of areas of tuber. A) Sax2015 Sig. dif. indicated by the following: * = against control, ● = against 12 mg / L, ◊ = against 32 mg / L. B) Sax2016 Sig. dif. indicted by: * = against control. Sig. dif. obtained via ANOVA, where indicated by 't' the sig. dif. obtained via t-Test two sample.*

Fluctuations in uptake (i.e. preference in Ca concentration) are possibly due too climatic (extremes of heat) and genetic conditions [32,67,68] that are beyond the remit of these trials and present the possibility of further investigation. To observe Ca uptake, the samples were segregated as described in 3.4. A high concentration of Ca is retained in the skin than in the flesh of the tuber, figure 37. It is expected that the skin of the tuber will contain a higher proportion of Ca compared to the rest of the tuber as this is the interactive surface to the rhizosphere. The second highest area would be the pith as this area of the tuber contains the xylem, where the Ca^{2+} is exclusive transported with transpiration as the main driving force for transportation [69,70]. It has been published that the main uptake of calcium occurs via the stolon root system and tuber rather than the main root system due to a more established xylem system [69], however Ca retention in the tuber is relatively

low due to low transpiration rates as tubers are surrounded by moist soil, therefore low transpiration rate occurs in the tuber [71].

Figure 37A and B, show the concentration of Ca through the tuber with figure 38 representing the percentage of Ca distributed through the tuber. Displaying percentage of mineral distribution allows observation of the transfer factor (TF) of the MONP [72]. The TF allows to establish the ability of the CaNP to biofortify the tuber [72,73].
Significant increase in Ca concentration in the skin was found in tubers fed with calcium ferrite.



	Control: Sax2016	Chempak : Sax2016	Control: Sax2015 (equiv. chempak 2016	CaNP+His . 12 mg / L: Sax2015	CaNP+His 32 mg/L :Sax2016	CaNP+His . 32 mg / L: Sax2015	CaFeNP+ His. (24:12 mg / L): Sax2015	CaNP+His 64 mg/L: Sax2016	His 32 mg/L:Sax 2016	His 64 mg/L: Sax2016
Skin / cortex	69.05	70.64	51.73	59.60	63.97	62.98	70.58	64.30	74.72	59.34
Tuber	30.95	29.36	48.27	40.40	36.03	37.02	29.42	35.70	25.28	40.66
Parenchyma / vascular ring	13.59	12.16			12.51			16.55	9.23	21.27
Perimedulla / medulla	17.36	17.20			23.52			19.15	16.06	19.38

Figure 33: Ca distribution (percentage) and comparison between trials Sax2015 and Sax2016

Observing the percentage Ca distribution through the tuber, figure 38, it becomes clear the application of CaNP+His. 32 mg / L, has the same distribution of Ca in consecutive years, therefore a consistent TF. It was also noted the His. 32 mg / L tubers had a higher Ca distribution in the skin than the CaNP counterparts. Amino acids increase the assimilation of minerals from the rhizosphere into the roots and the tuber, > 90 % are chloride, nitrates and other organic salts [32]. From this data, it can be suggested that the calcium oxide nanoparticle offers a more bioavailable Ca as is it transported more freely through the tuber into the flesh, particularly into the perimedulla / medulla region, where it is then transported throughout the plant.

4.4.2. Fe fortification in potato tubers hydroponic and compost propagation; H2015, Sax2015, Sax2016, Field2015 and Field2016.

From the data obtained from hydroponic and compost propagation, a discrepancy of optimal concentration of FeNP application was observed, figure 39. Tubers propagated with FeNP+His. 12 mg / L under hydroponic conditions obtained an increase of 55.60 %, with a significant decrease of Fe when fed with FeNP+His. 16 mg / L, suggesting a detrimental effect to the plant as the growth rate is decreased at this concentration. Sax2015 application of FeNP+His. 12 mg / L saw an insignificant increase of Fe content using ANOVA signal factor analysis nonetheless obtained a 26.71 % increase. A significant increase of Fe was found in the 16 mg / L, figure 39B, against all other applications including 12 mg / L ($p = 6.95 \times 10^{-6}$). This significant increase was repeated in the Sax2016 trial when compared against control plants and a 6.85 % increase against Chempak (comparable to 'control Sax2015').

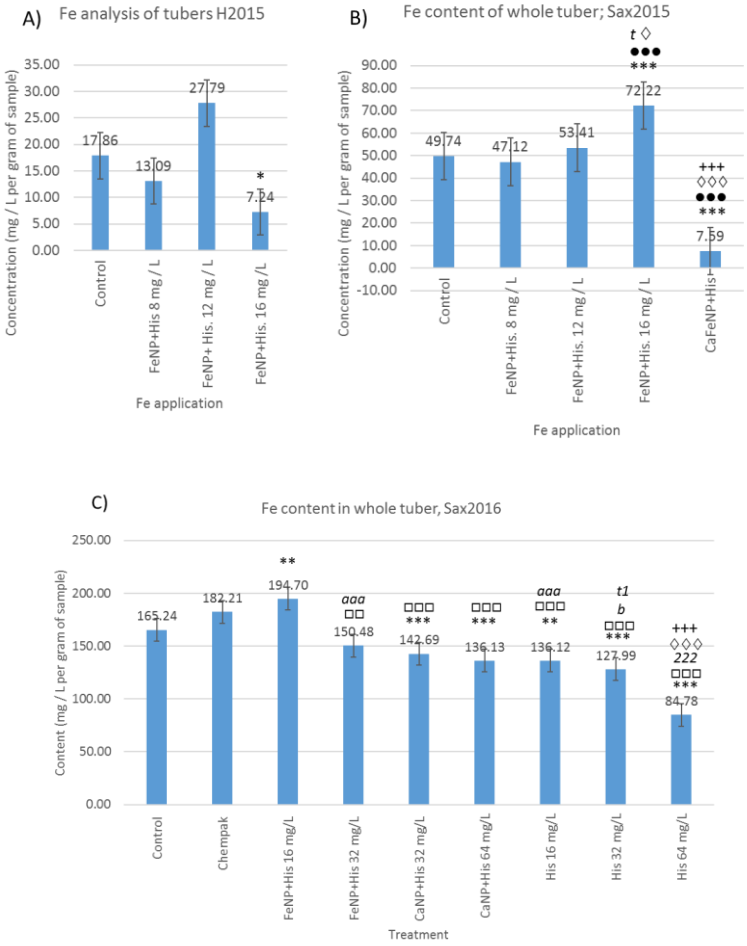


*Figure 34: Fe content of whole tuber. A) H2015; influence of MONP in a hydroponic system on the mineral content of tubers. Sig. dif. between 'Control' and 'FeNP+His.16 mg / L' = * B) Sax2015; Fe content of tubers propagated in greenhouse conditions in multipurpose compost. Sig. dif. indicated by the following: * = against control, ● = against 8 mg / L, ◊ = against 12 mg / L, + = 16 mg / L. C) Sax2016; Fe tubers cultivated under poly-tunnel conditions in multipurpose compost. Sig. dif. indicted by: * = against control, □ = against Chempak, a = FeNP+His. 16 mg / L, b = FeNP+His. 32 mg / L, 1 = CaNP+His. 32mg / L, 2 = CaNP+His. 64 mg / L, ◊ = His. 16 mg / L, + = His. 32 mg / L. Sig. dif. obtained via ANOVA, where indicated by 't' the sig. dif. obtained via t-Test two sample.*

Using a H_0 *"the application of FeNP did not affect the Fe content of the tubers",* the Fe content of tubers treated with FeNP+His and His equivalent concentration. From figure 39 38 C, it is shown the FeNP have a significantly increased amount of Fe, (16 mg / L, $p = 6.81 \times 10^{-7}$ and 32 mg / L, $p = 0.0148$), concluding the FeNP has fortified the tuber and

not the increased mineral assimilation amino acids can induce [29,74]. With these results the H_o is rejected.

The tubers treated with calcium oxide nanoparticles where also analysed for their Fe content to compare / observe any suppression of Fe. From figure 40, the data shows a significant suppression of Fe when the CaFeNP+His was applied, although a significant increase in Ca content was obtained, figures 36B and 34.

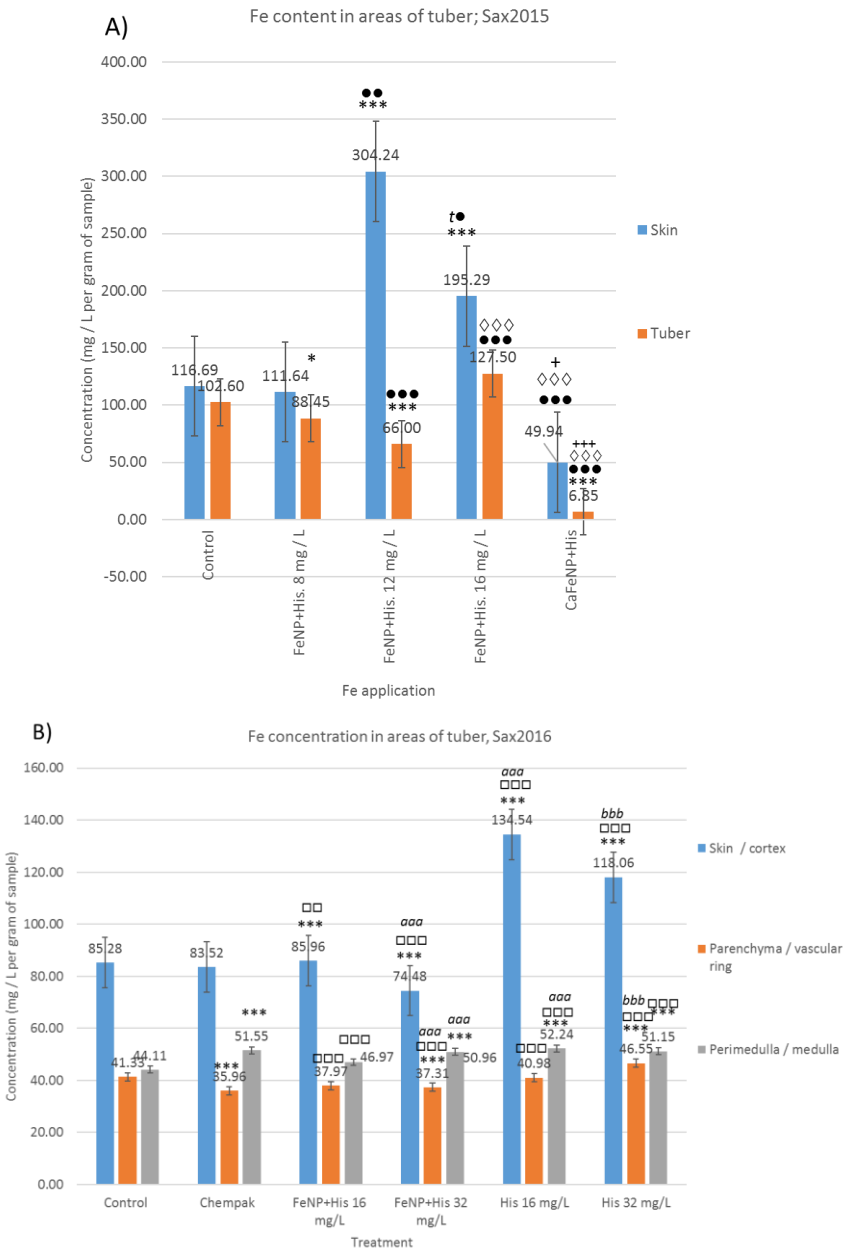
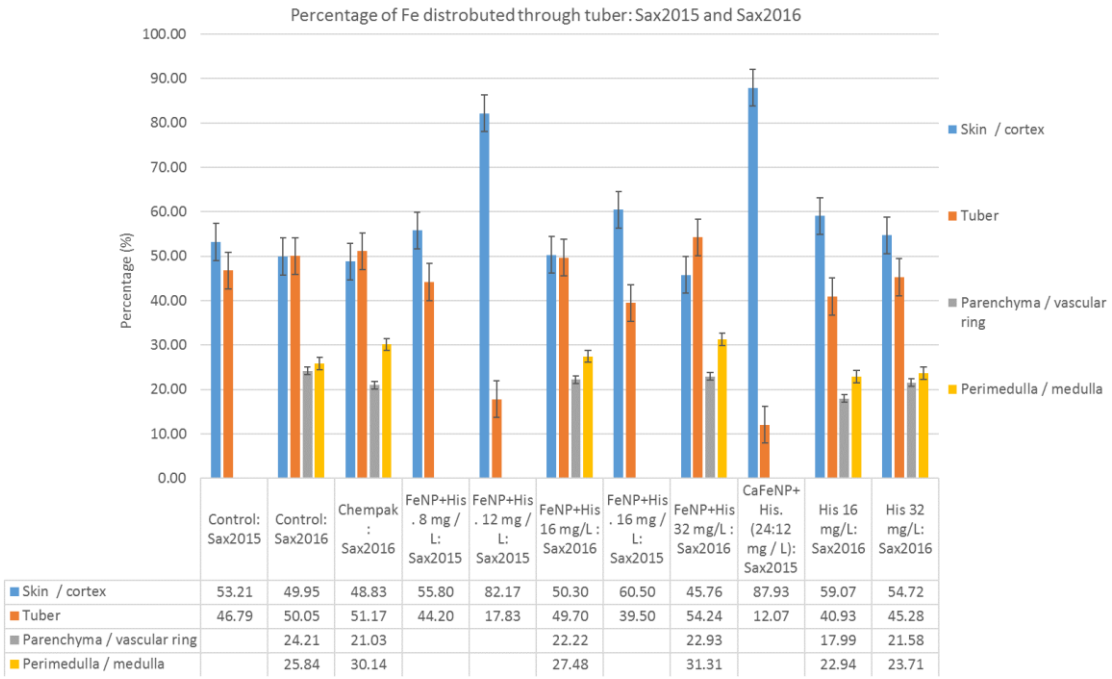


*Figure 35: Fe concentration in areas of tubers. A) Sax2015; sig dif indicated by; * = control, • = FeNP+His. 8 mg / l, ◊ = FeNP+His. 12 mg / L, + = FeNP+His. 16 mg / L. B) Sax2016; sif. dif indicated by; * = control, □ = Chempak, a = FeNP+His. 16 mg / L, b = FeNP+His. 32 mg / L. Sig. dif. obtained via ANOVA, where indicated by 't' the sig. dif. obtained via t-Test two sample.*

Iron concentration in the tubers treated with His. displayed a high concentration retention of Fe in the skin, 16 mg / L = 134.54 mg / L and 32 mg / L = 118.06 mg / L (figure 40) with 59.07 to 54.73 % (figure 41).



	Control: Sax2015	Control: Sax2016	Chempak : Sax2016	FeNP+His . 8 mg / L: Sax2015	FeNP+His . 12 mg / L: Sax2015	FeNP+His 16 mg/L : Sax2016	FeNP+His . 16 mg / L: Sax2015	FeNP+His 32 mg/L : Sax2016	CaFeNP+ His. (24:12 mg / L): Sax2015	His 16 mg/L: Sax2016	His 32 mg/L: Sax2016
Skin / cortex	53.21	49.95	48.83	55.80	82.17	50.30	60.50	45.76	87.93	59.07	54.72
Tuber	46.79	50.05	51.17	44.20	17.83	49.70	39.50	54.24	12.07	40.93	45.28
Parenchyma / vascular ring		24.21	21.03			22.22		22.93		17.99	21.58
Perimedulla / medulla		25.84	30.14			27.48		31.31		22.94	23.71

Figure 36: Comparison of Sax2015 and Sax2016 of the distribution on Fe through areas of tuber via percentage (%).

The distribution observed from 'FeNP+His. 32 mg / L' (figure 41) displays a phenomenon among the percentage distribution as the total tuber percentage, 54.24 % is higher than skin, figure 41, concluding a high TF. This data also displays a high proportion of the Fe located in the centre of the tuber (perimedulla / medulla), 31.31 % when applied at 32 mg / L.

Fortification of tubers propagated in collaboration with Branston (Field2015 and Field2016) show an increase in Fe content, figures 42 and 43.

Statistical comparison to control (T1) in trial Field2015 showed no significant difference (figure 42) for data obtained from midway (12 wap) and harvested (21 wap). When ICP data from midway T2 tubers (drench only application), the T2 tubers were found to contain highly significantly lower than T3 ($p = 7.88x10^{-6}$) and T4 ($p = 1.25x10^{-3}$). However, at the end of the trial, T2 tubers gained a highly significant increase in Fe content over T4 ($p = 6.16x10^{-3}$).

Highly significant Fe fortification were found in all treatments when comparing midway Fe content and harvested Fe content (figure 42). Generally, the foliar applications gained a reduced amount of Fe in tubers at the end of harvest (T3 = 67.58 mg / L and T4 = 55.57 mg / L) compared to drench only applied FeNP+His, 50 mg / L, T2.

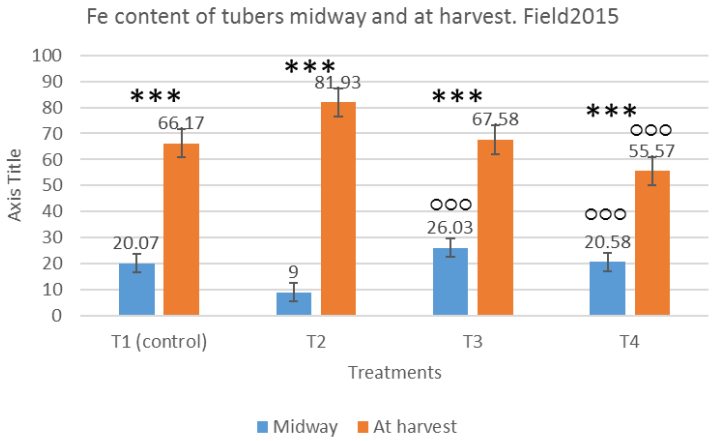


*Figure 37: Fe content of tubers from trial Field2015, propagating the red variety Mozart. Significant difference against T2 = o, between midway and harvest Fe content = *.*

As the drench only (T2) application of FeNP+His, Field2015 trial was deemed a successful fortification method, the trial was repeated in two separate sites (A and B) within 5-mile radius. Site A contained an increase in the loam content than B. Both sites were not deemed as Fe deficient, as with the previous site used in trial Field2015.
Two different varieties of potato were cultivated in both sites to observe the difference in response to an increased Fe availability [32]. Figure 43A. Both varieties of tuber increase in Fe content when exposed to FeNP+His., 50 mg / L, at planting through a drench application with Maris piper gaining a significant increase ($p = 0.0108$, 36.95% increase) with Inca bella gaining 6.41 % increase in Fe content.

From figure 43B, the influence in soil composition had an effect on the Fe content of the tubers, although with the application of FeNP+His., similar concentrations of Fe were obtained in both varieties. The increased loam at site A, increased the Fe in the control tubers in the Inca bella tubers, (6.38 mg / L, 13.27 %), but decreased the Fe content when compared to the sandy soil site B Maris pipers, (18.00 mg / L, 30.31 %) observing the preference of varieties to differing soil environments, table 14 [32].

Percentage increase in Fe content (%)

	Site A	Site B
Inca Bella	1.61	11.85
Maris Piper	68.56	14.91

Table 9:Percentage increase in the content of Fe between control and FeNP+His. application from trial Field2016

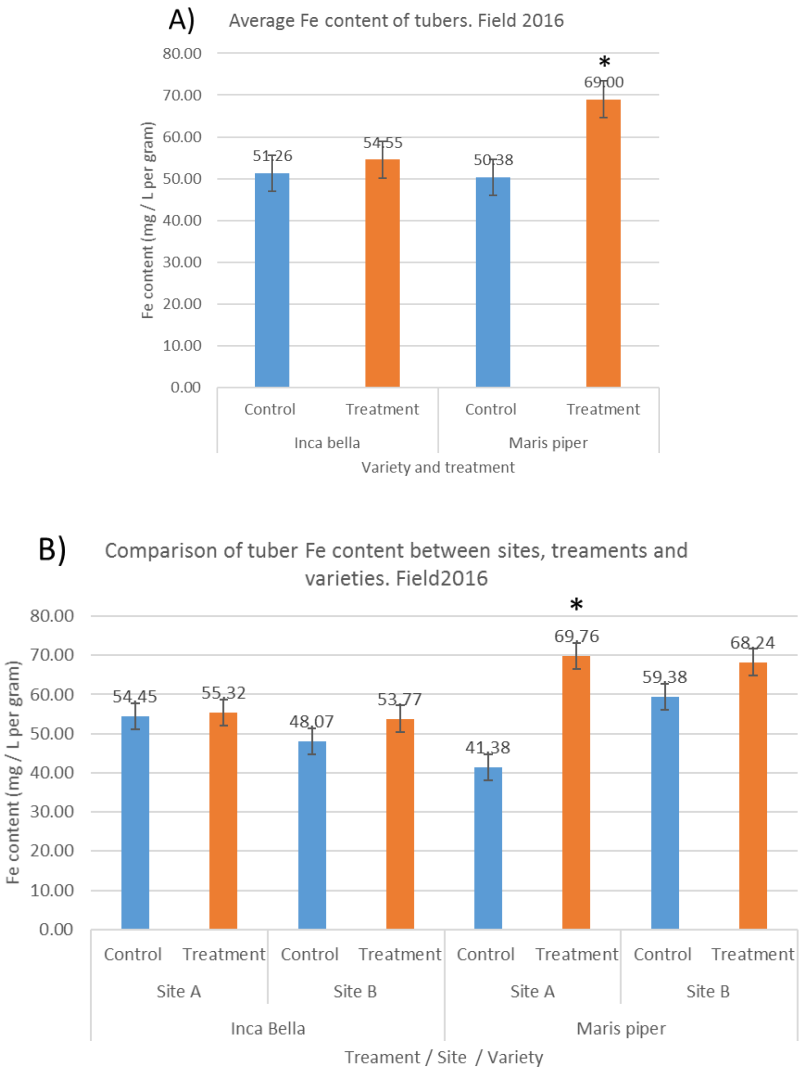


*Figure 38: Field2016 ICP data showing the Fe content of tubers. A) Average Fe content collated from both sites comparing control and FeNP+His. treated. B) Comparison of tuber Fe content between sites, treatments and variety. Significant differences between control and treatment are indicated by *.*

4.4.3. Zn fortification in potato tubers hydroponic and compost propagation: H2015, Sax2015, and Sax2016.

In both trials, H2015 and Sax2015 gained significant increases in Zn content. The 16 mg / L concentration produces a significant increase in Zn content in trial H2015 and a significant decrease was obtained in Sax2015.

In Sax2015 trial, figure 44, the tubers contained greater amount of Zn compared to H2015 tubers with no detrimental effect to growth rate.

A significant increase in Zn concentration by means of ZnNP+His. application, by passive or active transportation, with optimal fortification at 8 mg / L was found within the tubers Sax2015.

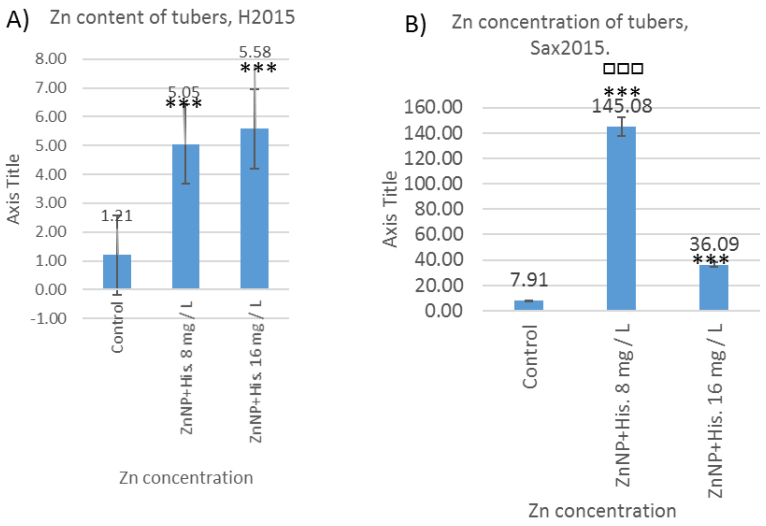


*Figure 39: Average Zn concentration of tubers. A) H2015, hydroponic propagation; * indicated the sig. dif. between control and ZnNP+His. applications. B) Sax2015, under greenhouse conditions with multi-purpose compost. Sif. dif. against control = *, between Zn applications = □. Statistical analysis via ANOVA single factor and ranked by p value as previously described.*

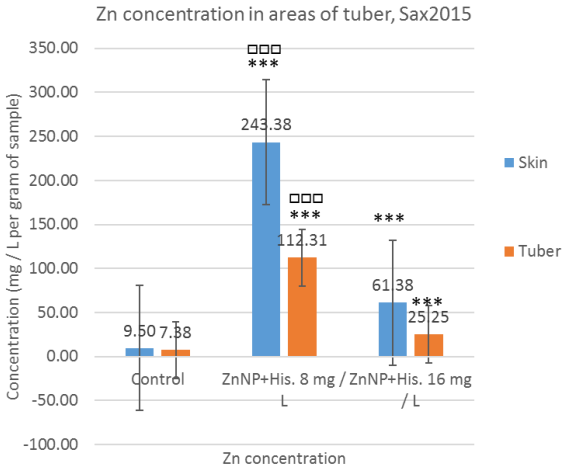


*Figure 40: Zn content of areas of tuber from Sax2015. Sif. dif. against control = *, between Zn applications = □. Statistical analysis via ANOVA single factor and ranked by p value as previously described.*

The application of ZnO nanoparticles have a positive effect on the biofortification of tuber due to significant increase from application ZnNP+His. 8 mg / L in both hydroponic propagation and in a compost media. Investigation in to the retention and aggregation of Zn in a compost / soil media is required as the interaction between ZnO and organic ligands in order to develop Zn fortification with ZnO nanoparticles.

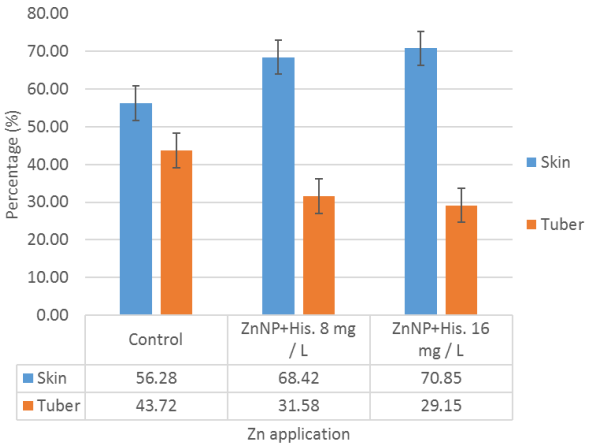


Figure 41: Percentage of Zn distribution in areas of the tuber.

4.5. **Retention of MONP in compost, Sax2015.**

The histidine coating of the nanoparticles, increases mobility through the strata due to the ability to suspend the nanoparticle and move with water. This allows passive diffusion into the tuber/root membrane through a concentration gradient. The amino acid coating provides a barrier to limit the mineral to complexing with ligands in the compost that would otherwise decrease availability. However, increased mobility and reduced ability to complex may lead to leaching of the MONP to the lower level (30 cm). From mineral analysis form the tubers, it was observed there is fortification from the application of MONP. With this in mind, two null hypothesis formed:
H_o^1; the amount of mineral at the depth of 5 cm is less than at 30 cm due to leaching when MONP+His. applied.

H_o^2; there will be no significant change in the concentration of minerals when MONP+His applied when compared to control at depths of 5 and 30 cm due to increased assimilation of minerals from compost.

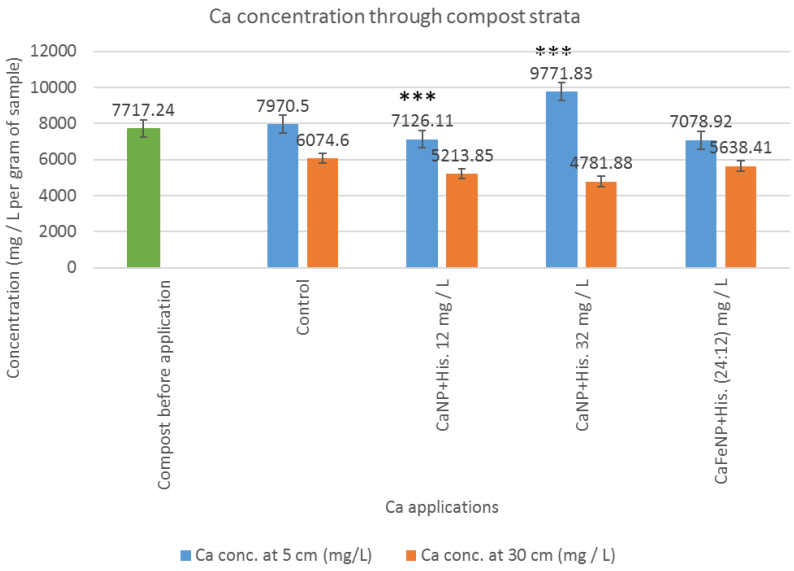


*Figure 42: Ca content in compost after harvest Sax2015. Using * to signify the sig.dif. between Ca concentrations at depths 5 and 30 cm with in the application using ANOVA single factor.*

From figure 47, there was not a sif. dif. between Ca concentrations at 5 and 30 cm, with a decrease of 1895.9 mg / L, nor between the Ca concentration between control and compost before the trial commenced (accepting H_o^2). The nature of the compost with reduced pH and increase of organic acids that increase the uptake of calcium, as with other minerals, are responsible for the reduction at 30 cm. The control propagation was fed with Chempak, as was the other Ca applications in Sax2015, thus increasing N and possible Ca phytoavailability.

There was no sig. dif. between control and Ca applications, however as figure 47 depicts, a sig dif. between 5 and 30 cm was found for applications CaNP+His 12 and 32 mg / L, therefore rejecting H_o^1. The application CaNP+His. 32 mg / L, increased Ca concentration at 5 cm by 25.60 % compared to 5 cm control. The application of calcium ferrite nanoparticles gained the highest concentration of Ca in the tubers harvested from Sax2015 than the application CaNP+His. 32 mg / L with a lower concentration of 12 mg /. There was a higher TF between in the skin of the tubers and compost which allows the conclusion that the reduced amount of Ca in the compost strata has increase the phytoavailability of Ca and thus taken up in the tuber.

From the analysis of the mineral content of the tubers, it was observed that the whole tuber optimal uptake of Fe was contained with the application FeNP+His. 16 mg / L with 12 mg / L producing the highest concentration in the skin giving indication that FeNP+His. successfully fortifies the tubers, whether from the Fe supplied by the nanoparticle and / or increased assimilation with the presence of histidine. This notion is supported by the significant decreases found when comparing control concentrations at 5 and 30 cm with the FeNP+His. counter parts concluding to reject H_o^2.

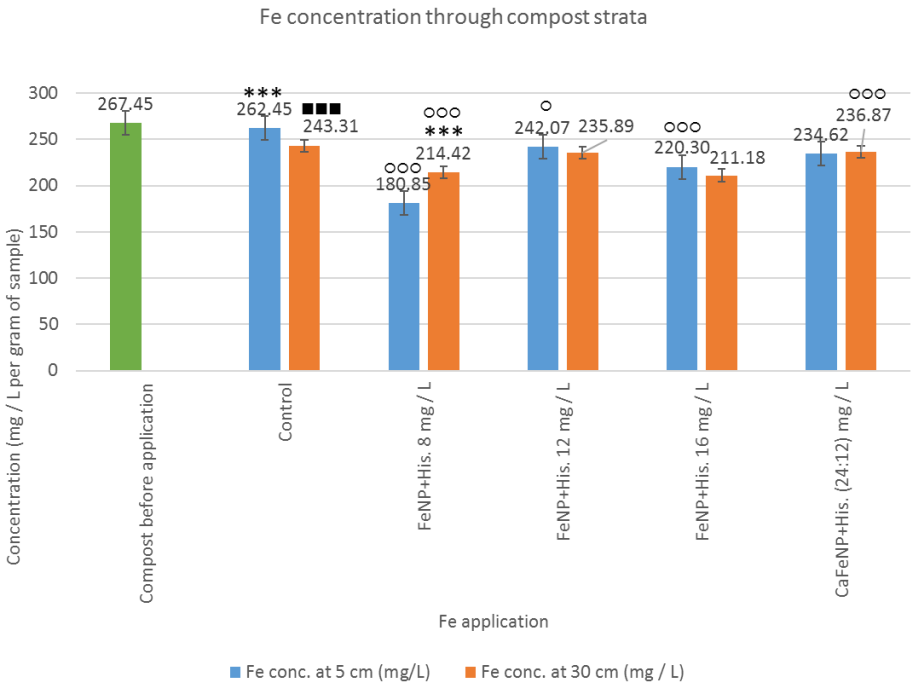


Figure 43: Fe content in compost after harvest Sax2015. Using ANOVA single factor significant differences where indicated '' between depths 5 and 30 cm within application,'■' against compost (control only tested), 'O' against control counterpart.*

A significant higher concentration at a depth of 5 cm than 30 cm was found with control with a significant decrease at 30 cm of the control against compost before application (figure 48). The decrease at 30 cm indicates the uptake of Fe around the tubers from the compost. Application of 8 mg / L produced an exception to the results obtained throughout this study (figures 47,48 and 49), with a significant reduction of mineral content at 5cm, figure 48. Due to significantly lower Fe concentration in the 5 cm when compared to control and compost before the trial, this suggests Fe released from complexes in the soil and subsequent leaching into the lower strata. The Fe content increases by 33.57 mg / L at 30 cm, accumulating around the areas of the tubers, concluding the acceptance of H_o^1.
Applications 12, 16 mg / L and CaFeNP+His. show Fe retention throughout the strata of the compost. The FeNP+His. application 16 mg / L tubers contained the most Fe (72.22 mg / L) with 12 mg / L application retained a significant amount in the skin, figure 49. This is reflected in the compost Fe content as the applications of 12 and 16 mg/ L decreased in 30 cm compared to the 5 cm concentration, indicating retention of Fe at 5 cm and utilisation of minerals in the tuber region. These concentrations were lower than the control counterparts, reaffirming uptake of Fe from the compost. Concentration results from CaFeNP+His. compost, shows a decrease over control and compost before applications.

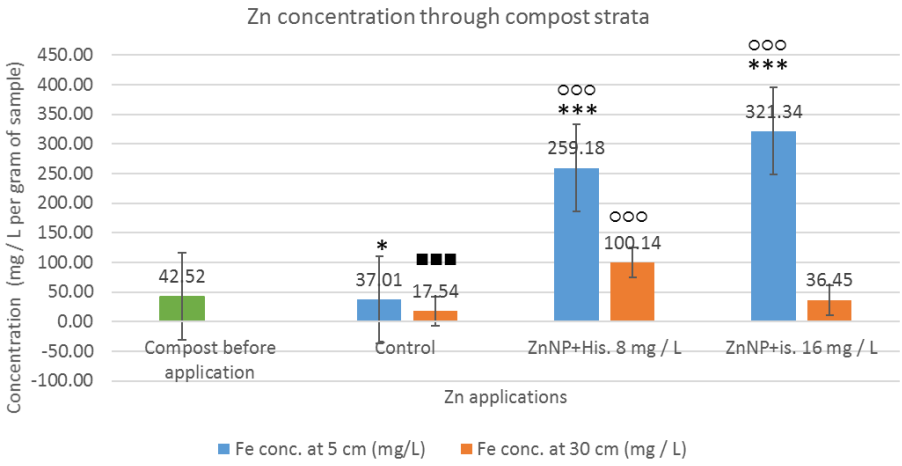


Figure 44: Zn content in compost after harvest Sax2015. Using ANOVA single factor significant differences where indicated '' between depths 5 and 30 cm with in application,'■' against compost (control only tested), 'O' against control counterpart.*

Comparing the Zn content of tubers from figure 46 and compost Zn data, figure 49, the application of 8 and 16 mg / L produced significantly fortified tubers over control. The concentration of Zn through the application of zinc oxide nanoparticles resulted in a significant increase of Zn at the depths of 5 and 30 cm depth, therefore rejecting H_o^2. The data collected indicated highly significant decrease in the content of Zn at 5 than 30 cm following the pattern found for Ca and Fe concentrations, with exception of FeNP+His. 8 mg / L thus rejecting H_o^1.

4.6. **Uptake and retention of Fe using 59Fe isotope.**

Using the radioactive isotope ^{59}Fe, FeNP+His. and FeEDTa were synthesised and applied to the compost as a solution at a concentration of 12 mg / L. Using serial dilutions of the stock solution of the FeNP and FeEDTA, MBq was converted into mg / L, figure 50.

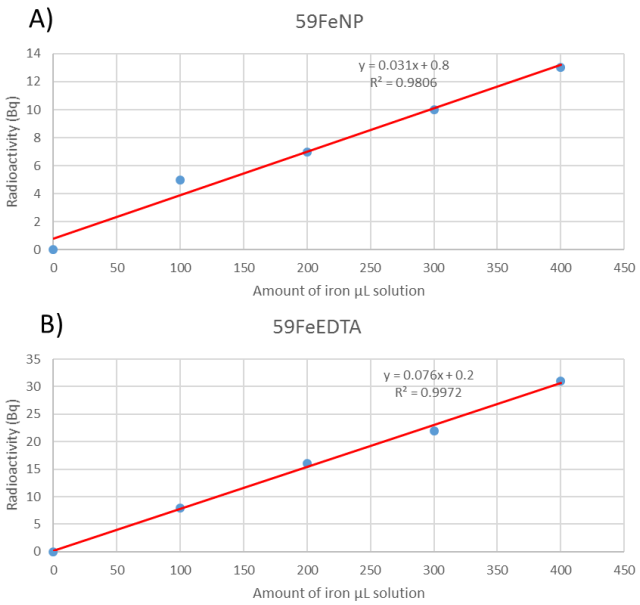


Figure 45: Calibration of radioactive 59FeNP and 59Fe-EDTA to determine Fe content using Hidex AMG Gamma Counter measuring the gamma reading (MBq).

Using ANOVA single factor statistical analysis, with a p ranking as follows; < p = 0.05 *, < p = 0.01 ** and < p = 0.001 ***, all data collected from FeNP+His showed a '***' of significance over Fe-EDTA concluding that the nanoparticle retained in the compost at a highly increased amount than the Fe-EDTA., figure 51A. Figure 51B, demonstrates a 578.4 fold increase in the amount of iron in the tubers propagated in the trial treated with FeNP+His application over Fe-EDTA. The amount of Fe distributed through the stem of the plant, figure 51C, was significantly higher for the application of Fe from the nanoparticle over the chelate. The Fe-EDTA distribution shows a decline in Fe content progressing up the potato plant stem. The Fe content of the stems from application of FeNP+His display a high Fe content at the lower stem like the Fe-EDTA, however, the top of the stem contains 109.07 % (1.33 mg / L) more Fe than mid stem. Due to radioactive regulations limiting the contact with the radiation and plants, the growth rates were unable to be observed. The increased Fe concentration suggests the escalated production of chlorophyll, which Fe plays a key part, suggesting new leaf development.

Repetition of the experiment to include other Fe-chelates over a larger sample number would enable a comprehensive view of the increased uptake, utilisation and retention the iron oxide has above Fe-chelates and $FeSO_4$. It was noted that during the experiment that the foliage of all participating plants where suppressed or damaged due to the strength of the gamma and beta radiation produced from ^{59}Fe. The initial dosage of 1 mCi was deemed to be too strong even with the occurrence of two half-lives (28 days), due to laboratory and personnel availability, it is deemed that a stock sample from which the FeNP and Fe-chelated would be synthesised would be 500 µCi.

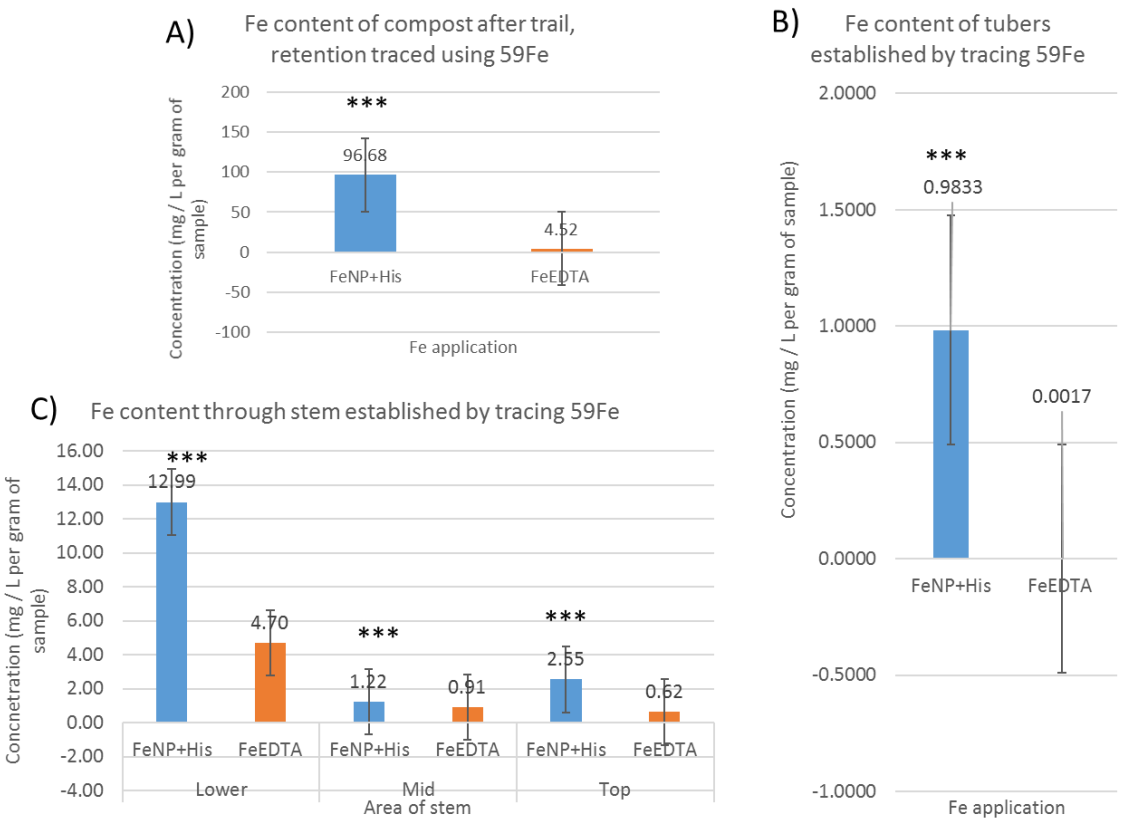


Figure 46: Comparison of the Fe content recorded from the MBq reading produced from 59Fe isotope and converted into mg / L per gram of sample. A) Fe content from the growth media, multi-purpose compost, after the trial was completed. B) Fe content from tubers propagated in trial and C) areas of stem sampled at the end of the trial.

4.7. Investigations into the antibacterial properties of MONP

- Antibacterial properties of CaNP+His solution (CaO and $CaFe_2O_4$)

To establish the antibacterial effects of Ca upon a range of bacteria, a sample of wash water from a potato processor, Produce World, Sutton Bridge was used as the source. From figure 52, it can be observed that the His and CaFeNP+His gained significantly lass bacteria (CFU / mL) that control and CaNP+His. The treatment of CaNP had a very slight decrease in the number of CFU / mL observed, however, the addition of the Fe element may increase the antibacterial properties of calcium [76,77], thus requiring further investigation. The solutions were buffered prior to the waste wash water, therefore eliminating the acidic influence of the amino acid, histidine.

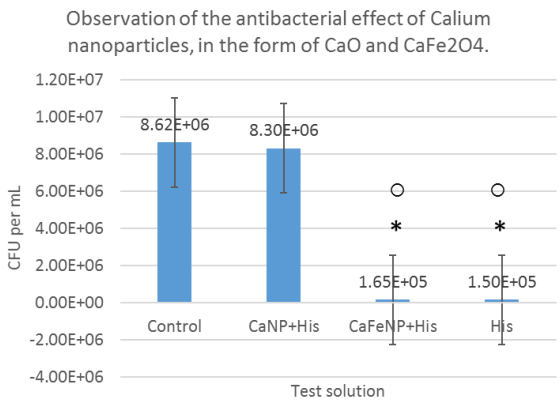


*Figure 47: The antibacterial effect of two forms of Ca nanoparticle as an antibacterial agent against soil bacteria. Sig. dif indicated between control = *, against CaNP+His = O.*

4.7.1. Tuber inoculation using PCA soak to tubers pre-soaked with MONP.

A number of tubers from were collected from same storage conditions at CSRF, washed in water, dried then soaked in CaNP+His and CaFeNP+His. 100 mg / L, table 15, with 3 repetitions from each tuber. During periods of prolonged contact with moisture, the pores of the tuber open allowing the passage of PCA into the tuber. Utilising this period, a 'soak' method of fortification with the calcium oxide and calcium ferrite was utilised. The nano size of the particulates will increase the transfer into the tuber as they are sub size of the membrane pores [26]. When membrane pores dilate in the soak, this will increase the uptake of nanoparticles but also increase the permeation of the bacteria into the cortex and parenchyma. Increased Ca concentration of the cells of the treated tuber will counteract any bacterial infection via the enhanced structural integrity of cell walls and membrane [77] preventing cellular damage from bacterial colonisation.

Soak treatment	*No. tubers per test*	*Length of soak period*	*PCA soak inoculation*	*Samples*
No soak	5	N/A	2 hours	Swab & peel 3 reps of each On LB plates (3 plate reps per sample)
Dist H20		Soak period of 24 hours		
CaNP+His.				
CaFeNP+His.				
FeNP+His				
His				

Table 10: Conditions for the comparison of MONP against coating for antibacterial properties against PCA.

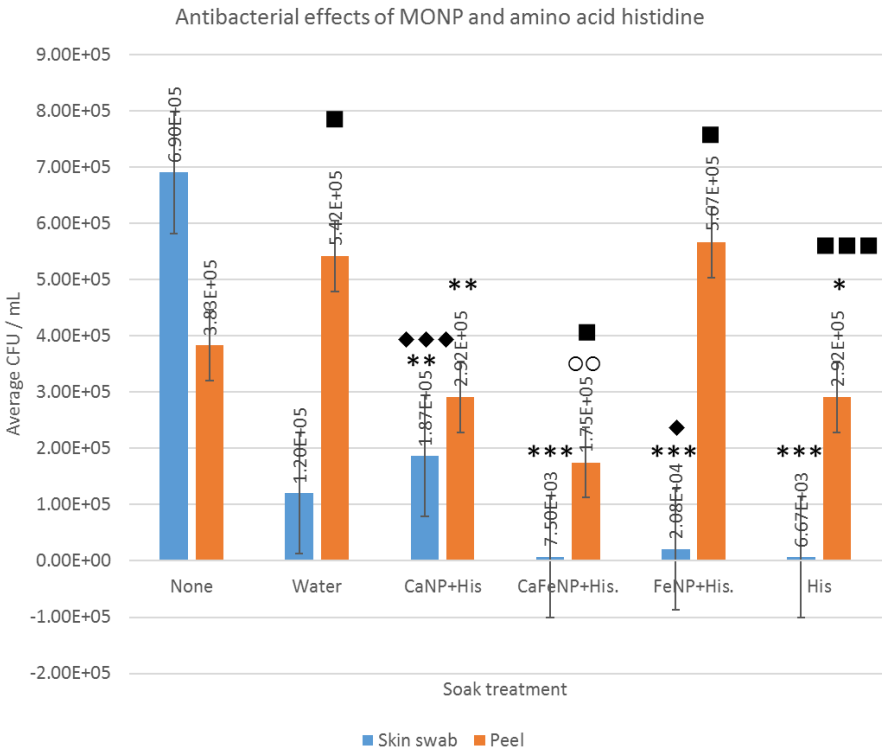


*Figure 48: Antibacterial effects of MONP and histidine on PCA. Significant differences obtained from ANOVA single factor statistical test between non-soaked and other treatments are allocated= ○, against water = *, against His. = ◆ and swab against peel counterpart = ■.*

As on observed from figure 53, no significance was found between 'no soak' and other treatments even though the average PCA CFU / mL is considerably higher that other data collected. The action of the soak in itself will decrease the surface bacteria resulting in the reduction of CFU / mL from $6.90x10^5$ (none) to $1.20x10^5$ (water soaked). As expected the action of soaking the tuber enabled the bacteria to transport into cortex and parenchyma region of the tuber, as the peel data increase from $3.83x10^5$ CFU / mL (none) to $5.42x10^5$ (water soaked), figure 53.

CaNP+His. treated tubers gained a moderate significant increase in PCA in the skin swabs ($p = 4.98x10^{-3}$) against control, however, the peel PCA CFU / mL gained significantly less ($p = 2.58x10^{-2}$). The positive charge of the CaNP may 'attract' the bacteria to the skin surface and restrict bacterial progression into the cortex as a supplementary effect to the increased integrity of the cellular structures [26,77,78].

Treatments CaFeNP+His, FeNP+His and His., all gained highly significant decreases in PCA concentration against water soak. Fe causes oxidative stress to the bacteria even if magnetite (Fe_3O_4) is fully oxidised to maghemite (γ-Fe_2O_3) [79,80,81] resulting in the decrease of PCA on the surface of the skin. The absence of Ca in the FeNP+His. treatment saw an increase in the PCA CFU / mL on the peel sample from $1.75x10^5$ (CaNP+His.) to $5.67x10^5$ (FeNP+His). The application of His. observed a highly significant decrease in PCA on the skin surface suggesting the acidic nature of His. may be responsible for the CFU / ml decrease.

4.7.2. Comparison of the effect of MONP treatment on tubers; with and without PW. W H_2O used as a bacterial source.

The potential anti-bacterial effect of MONP; CaNP+His. and CaFeNP+His. (200 mg / L) were compared against the commercial antibacterial application, Produce wash (PW), table 15. A second investigation observed the interaction of PCA with tuber treated with MONP when previously washed with PW, table 16.

For control tubers '1' and '1a', soaking in distilled water to be in conjunction with 'soak treatment' of CaNP and CaFeNP. During periods of prolonged contact with moisture, the pores of the tuber open allowing the passage of PCA into the tuber. Utilising this period, a 'soak' method of fortification with the calcium oxide and calcium ferrite was utilised. The nano size of the particulates will increase the transfer into the tuber as they are sub size of the membrane pores [5]. When membrane pores dilate, this could increase the uptake of nanoparticles. Treatment applied to 1 - 3 is a repetition of conditions in the previous study, adjustments to the concentration (100 to 200 mg / L) and excluding FeNP+His. and His. as the commercial focus would preferably be on a Ca application.

	Conditions	*Washing*	*Treatment (24 hr soak) @ RT*	*PCA soak 2 hours @RT*	*Swab and peel samples taken onto LB plates (2 reps per sample)*
1	Control	Water	Water		
2	Water/CaNP+His.	Water	CaNP+His		
3	Water/CaFeNP+His.	Water	CaFeNP+His		
1a	PW/ no treatment	PW	Water		
2a	PW/CaNP+His.	PW	CaNP+His.		
3a	PW/CaFeNP+His.	PW	CaFeNP+His.		

Table 11: Washing applications and post-wash treatment with application of CaNP+His. and CaFeNP+His

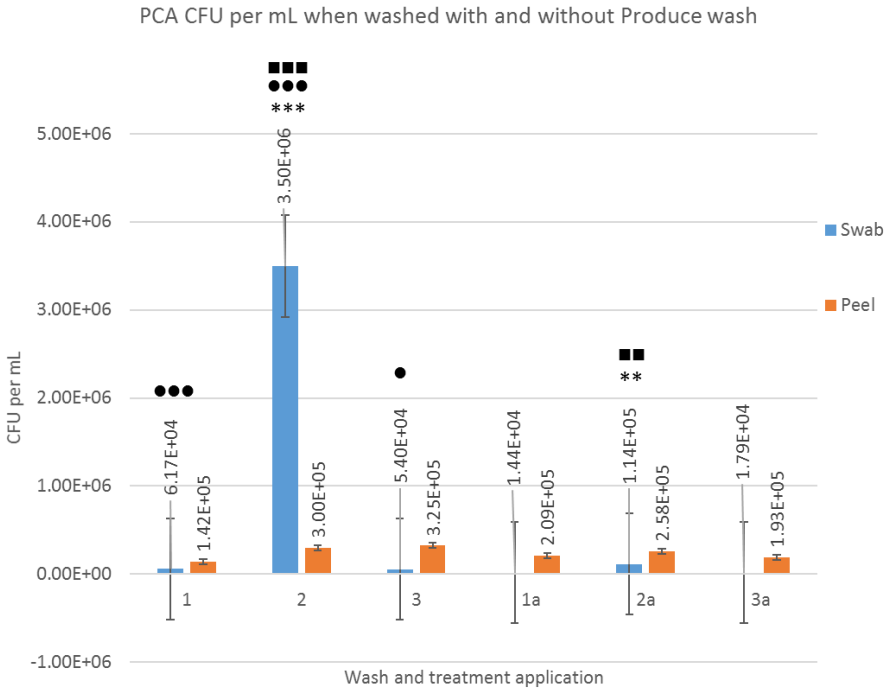


*Figure 49: Comparison of PCA (CFU per mL) obtained from skin swab and peel to observe the action of CaNP and CaFeNP with the application of Produces wash before application. * = against 1,1a. □ = against 'a' counterpart.*

With a two null hypothesise; H_o^1= "*the use of Produce wash does not decrease the amount of PCA, CFU per mL*" H_o^2 = "*application of CaNP+His. or CaFeNP+His. does not decrease the amount of PCA, CFU per mL*". Using the statistical analysis test, ANOVA single factor and ranking the *p* value (< p = 0.05, *; < p = 0.01, **; < p = 0.001, ***) the data was analysed.

There was a significant increase in the concentration of PCA in swab samples taken from potatoes that were washed with water only (figure 54) indicating the use of PW reduces the presence of PCA on the potato skin surface, rejecting H_o^1. No significant difference was found in the peel data, concluding the PW or nanoparticles have any reduction in the passage of PCA into the tuber, accepting both null hypothesis.

Application '2' obtained a significant increase in the amount of PCA on the skin swab but no difference was found between peel control, '1a' or '3a' suggesting the PCA does not transfer through the skin. H_o^1 is rejected for application '2' due the significant increase was obtained, and H_o^2 is accepted for application '2a' as there was not a significant difference obtained when compared to '1a'.

The application of CaFeNP+His., '3' and '3a' did not gain any significant difference when compared to controls, '1' and '1a', although a significant decrease (p = 0.0248) was found when PW was used.

5. DISCUSSION

5.1. Effects of MONP on crop development and fortification

Interestingly the application of FeNP+His in the trial 'Field rep 2016', observed an increase in growth rate shortly after application, figure 25, indicating an influence of the FeNP+His. Further investigation in the effect the FeNP+His or His has upon the growth rate and the timing of application (in the life cycle) is required in line with yield and DM%, speculating that increased energy going into vegetative production could hamper the tuber formation and loading.

The hydroponic application of ZnNP+His treated potatoes are significantly suppressed by the presence of ZnNP with increases of 13.73 mm and 13.18 mm, ZnNP+His 8 and 16 mg / L respectively. Ca+HisNP 12 mg / L did not grow as rapidly as expected with a height increase of 31.43 mm when compared to the increase of 216.63 mm obtained by Ca+HisNP 32 mg /L (figure 21a). Compared to application to compost sig. dif. of stem heights, figure 22 and 23. CaNP+His 32 and 64 mg / L has a significant increase in height over control, Chempak and the His. equivalent suggesting an influence in the presence of CaNP. This is supported by figure 24a where there in an increase in growth rate in stems treated with CaNP+His 32 mg /L.

Yield analysis using ANOVA single factor analysis, from trial Sax2015, a significant increase between control plants overall average weight (OAW) in grams, and treatments, CaNP+His 12 mg / L ($p = 0.01$), CaFeNP+His ($p = 1.43 \times 10^{-4}$), ZnNP+His. 8 mg / L ($p = 3.21 \times 10^{-5}$) was found. Treatments CaFeNP+His and both ZnNP+His did not produce any sub 30 mm tubers where as FeNP+His did.

Treatments CaFeNP+His and both ZnNP+His did not produce any sub 30 mm tubers. Due to the harvest occurring at 14 weeks, approximately 20 days short of commercial harvest, the occurrence of <30 mm tubers would be expected as these tubers would go onto to produce 'salad' crop. The absence of these bud tubers (sub 30 mm) indicated the plant has halted tuber initiation early into the growth cycle. The presence of ZnNP or CaFeNP did not supress the vegetable development of the treated plants, nor did individual application of FeNP and CaNP supress development as previous discussed. This anomaly required further investigation in the form of repetition of the trial on a larger scale such as a field trial to observe an increase in environmental factors supress or increase this response. Investigation in to possible suppression of signalling pathways involved in tuber formation from the increased concentration of Zn, Ca or Fe in the rhizosphere, or the gene signalling involved in the uptake and transport in the plant, i.e. the expression of ZIP genes and ferritin [29,82].

The influence of FeNP+His upon the yield of larger tubers (<30 mm) was found to be significant in Sax2015, Sax2016, Feload2016 and an increase in the number of tubers harvested in Fieldrep2016. Field trials 2015 and 2016 did not observe any significant yield increases or decreases, but increase the DM %, which is of more economic importance to potato producers and production [36,64]. The application of CaNP+His at 12 and 32 mg / L, gained similar DM % as control, whereas the application of CaFeNP+His. (24:12 mg / L), ZnNP+His, 8 and 16 mg / L gained significantly less DM%. The application of ZnNP+His. 16 mg/ L did increase DM % over 8 mg / L; therefore, it would be of interest that investigations included increased concentrations of ZnNP+His to investigate increasing the DM% to improve the fusibility of a Zn fortified potato in the commercial environment.

5.2. **MONP application as a fortification**

The trials conducted here in found an increase in mineral content when applied to potato, as a solution to the soil and foliar application. The optimal concentration of MONP+His differed between varieties and with different trial environments. Fluctuations in uptake (i.e. preference in Ca concentration) are possibly due to climatic (extremes of heat, hydration) and genetic variation [32,68,83] that are beyond the remit of these trials. Increased collaborative work with commercial growers over a number of seasons would substantiate the influence of the environment (i.e. field trials) and delivery methods (hydroponic systems for tomato production, pellet or solution delivery in commercial potatoes cultivation).

Tubers gained significant amount of Ca in the flesh of the tuber and other Ca areas of the tuber (figure 37a and 37b). As observed, the Ca content is significantly lower than other applications of MONP, leading to the suggestion that the Ca concentration is at a phytotoxic level and retained in the skin to avoid cellular damage. Sax2016 trial contradicts this, as the Ca concentration in the flesh areas of the tuber obtain significant increases in Ca over 'control' and 'Chempak' treatments, especially in the perimedulla / medulla region where transportation to the rest of the plant occurs [32].

It is well documented that high content of Ca in calcareous soil limits the reduction of Fe^{3+} and uptake of Fe^{2+} causing the deficiency chlorosis [4,10]. The high pH of calcareous soils reduces the solubility of iron oxides by reducing H^+. Ca may be transferred by mass flow into the tuber / root system and accumulates in the rhizosphere, consequently calcium carbonate precipitation, with the formation of bicarbonate when increased CO_2 is, produced by a developing root system [75,86,87].

$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + HCO_3^-$

Plants suffering from chlorosis display stunted growth, yellowing of the vein area of young leaves and a significant reduction in yield. No yellowing or discolouration of the leaves treated with CaNP or CaFeNP where observed. Average height increase was 9.95% above control, figure 22 and table 8 showing no detrimental effect to the plant when fed CaFeNP. There was a decrease in the average number of tubers per plant, however, the average tuber weight was significantly higher than control, figure 27. As previously mentioned, tuber initiation was shortened, signified by the lack of < 30 mm when harvested. Therefore, with these number of contrasting results it is difficult to say without further investigation that the application of CaFeNP would course chlorosis or be an application to further improve the Ca fortification.

Due to the nanoparticles ability to passively enter the skin via the pores in the cell wall [26], plus amino acids increase the assimilation of nitrogen and chelation of metal present in the rhizosphere, increases uptake of FeNP in the skin [29,85]. Due to the high Fe^{+2} intake, it is possible that the excess Fe is retained as the protein ferritin to prevent cellular oxidation damage until it is chelated and transported via protein transported to organelles that utilise the Fe^{2+} [10,85].

Data from segregated areas of the tuber displays the translocation and potential utilisation of Fe sources from the nanoparticle throughout the plant. Figure 40A, demonstrates the high proportion of Fe is retained in the skin [32]. The application of 'FeNP+His. 16 mg / L' in both trials significantly increased the Fe concentration in skin and tuber over 'control' and 'Chempak' concluding the FeNP+His. is travelling thought the pores in the cell wall. However, when the data is displayed in percentage distribution to observe TF, a decrease

in the amount of Fe in the tuber areas differs between trials. This is possibly due to variation in climatic conditions or genetic variation [32] that are beyond the control of the trial conditions.

Both trials using compost as the cultivation media (Sax2015 and Sax2016), noted a tolerance to the higher FeNP+His concentration. The composition of the compost media enables a retention of the Fe due to varying number of composites (i.e. sand, clay and organic matter) found in soil and compost. These constituents differ in negative charge, which attracts the positive charge of the Fe^{2+} and Fe^{3+}, thus enabling a buffering effect to the tuber and root system [84]. In a hydroponic environment, the reduced retention ability of the pebbles, exposes the tuber and root system to more readily to the nutrients, in theory enabling increased uptake.

The foliar applications appear to inhibit the Fe uptake from the soil source suggesting the Fe from the foliar application it utilised by the leaves in the process of chlorophyll production, reducing the requirement to take up Fe via the root system thus reducing the amount of Fe passing and stored within the tuber.

The significant increase in Zn content in tubers increased with increasing ZnNP+His. application was noted in H2015 suggesting a successful fortification application, however, the foliar growth was severely stunted then treated with ZnNP+His. leading to question the phytotoxic effect of ZnNP in a hydroponic system. This is due to the additional Zn present in the compost as the only source of Zn in the hydroponically propagated tubers was ZnNP+His. The decrease of Zn content at 16 mg / L in Sax2015 could be explained by published investigations into the uptake in ZnO nanoparticles presented the rapid aggregation of ZnO in an aqueous solution when in the nutrient solution is not continually agitated as in a hydroponic system [80]. The increased size of aggregated ZnONP into the μm range, decreases the bioavailability as the particles are larger than cell wall pores [26,89,90]. Nano-scale pores 5 – 20 nm [26,88] located in the cell wall, allow the passive transportation of small molecules (< pore) while limiting the passage of larger modules [89]. Zinc oxide nanoparticles also bind strongly with various organic ligands present in compost and soil, as Fe [91], therefore, an increased concentration of ZnNP+His. along with the effects of nanoparticle aggregation, may have contributed towards the decrease in fortification of the tuber as observed in figure 44B.

5.3. **MONP in the environment.**

The application of FeNP+His. allows the delivery of both Fe^{3+} and Fe^{2+} as a stoichiometric ratio of 2:1 (Fe^{3+}/Fe^{2+}) [56] allowing a duel delivery of Fe that is phytoavailable immediately (Fe^{2+}) and a more stable Fe supply (Fe^{3+}) [3] that will not be as readily complexed as Fe^{2+} but available to the plant when Fe^{3+} is reduced in the rhizosphere via a proton pump mechanism [3,56]. The amino acid coating prevents the formation of insoluble complexes with retention in the growth media to allow slow delivery of bioavailable iron.
With increased mobility, it was questioned if excess nanoparticle will be leached through the strata with possible effects to the environment, in particular to watercourses, hence the requirement for this study.

The mineral content of soil is dependent of pH, organic matter and clay content, weather conditions and composition of parent material [12,92,93,94,95] and exist as free ions or complexed with minerals or organic surfaces, soluble compounds or as precipitates [12,92,93,94,95]. Free metal ions that are released or added via fertiliser, the ions interact with the charged particulates that may form weak complexes through cation exchange or

strong bond through ligand exchange. The associations these ions form largely depends on the nature of the ion and absorbing surface [92]. Metal ions of calcium, iron and zinc (Ca^{2+}, Fe^{3+}, Fe^{2+}, Zn^{2+}) are unavailable as they form strong bonds with clay and organic matter in the form of oxides and hydroxides binding the metals into the soil / compost matrix [10]. Insoluble complexes are unable to move through the matrix to the root / tuber to compost interface where reduction in the pH enables chelation and uptake.

Due to the low mobility of Fe in soils due to the nature of Fe being readily oxidised to form salts and highly insoluble oxides and hydroxides as follows [75]:

$$Fe^{3+} + 3(OH)^{-} \leftrightharpoons Fe(OH)_{3\ (solid)}$$

Manly Fe applications use salts, such as $FeSO_4.7H_2O$ and Fe-chelates to increase soluble Fe and hence the availability to plants particularly in calcareous conditions. Salts are extremely soluble and easily leached through the soil [75], therefore only used as a sort-term delivery. Chelates have been used since the early 1950's, as they have a high affinity constant to form a highly stable complex, delivering Fe at a reduced rate than $FeSO_4.7H_2O$ [46,47,52,96].

Ethylenediamineteraacetic acid (EDTA) is a potentially hexadentate chelating ligand (figure 55) [47,49] with each N contains a free pair of electrons and the molecule possesses four acidic hydrogens [47,49]. Other chelating agents include HEDTA, 2-hydroxyethylenediaminetriacetic acid; DTPA, diethylenetriaminepentacctic acid; EDDSA, ethylenediaminediscuccinic acid and IDSA, iminodisuccinic acid that are applied either as a foliar or root solution to increase Fe availability [50]. EDTA along with other chelates are used as a metal 'stripping agents', in the form of a treatment method to remove heavy metals from water courses due to its rapid strong chemical bond [51].

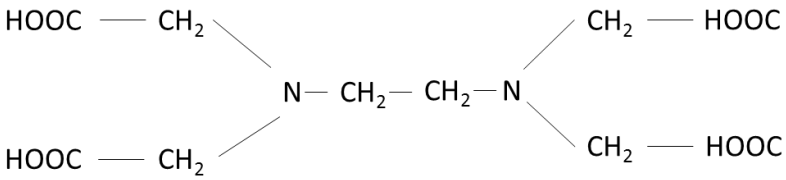


Figure 50: Schematic structure of ethylenediaminetetraacetic acid (H_4EDTA).

Published data from Shenker and Chen (2005) [52] observed Fe-EDTA had an increased stability constant (K_{app}) above other Fe-chelates, table 17, especially for Fe^{2+}. is the most commonly used chelating agent. However, 81% of soil applied Fe-EDTA has been shown to leach and lost the surrounding environment, rendering the availability of Fe as poor [53].

Fe-Chelate	Log K_{app}	
	Fe^{2+}	Fe^{3+}
EDTA	22.3	11.4
HEDTA	20.3	9.5
EDDHA	24.9	5.3

Table 12 Adapted from Shenker and Chen, 2005; comparison of Fe-chelates and stability constant (K_{app}).

The increased mobility of the FeNP due it the histidine coating, plus nano size of the particles, allows the passive movement of Fe into the cell like Ca^{2+}. The coating provides

a barrier to chemical binding, however, this feature has the potential to leach through the strata.
$Ca^{2+}$is a large divalent cation in contrast to Fe and Zn ions [14] and moves in conjunction with water when free, however, this a rare occurrence as it forms a tight bond with particulates so much that Ca leaching through the soils strata does not normally occur [12]. Unlike other minerals such as Fe and Zn, Ca^{2+} passively diffuses into the root / tuber via a gradient caused by transpiration in the leaves [12,14,71].
Data obtained for the growth media during the harvest of Sax2015 trial, the high organic matter composition of the multi-purpose compost naturally has a lower of pH of 5.5 than soils (e.g. from Branston field trial a pH of 6.5 was obtained). Due to an increase in organic acids from the increased organic material, minerals increase in phytoavailability via microbial decomposition [94].

A possible explanation for the increased mobility of Fe when supplied as FeNP+His, is possibly be due to a balance of the amino acid, histidine and the FeNP. Histidine could increase nitrogen assimilation and metabolism of the root and tuber, thus increasing mineral availability [28,29]. The concentration of iron released and Fe supplied by the FeNP is inadequate, or possible leached into lower strata (> 30 cm), to gain significant increase over control tubers. Another possible explanation could be due to the nature of the compost. Compost varies in composition and to overcome the differentials ten replicate were taken per sample. In hindsight more replicates are required with increased samples taken in a border range of the strata.

Free Zn ions are bound in the soils matrix similarly to Fe [97] and thus highly dependent in the pH of the growth media. Normality the Zn content of non-polluted soils is approximately $3 \times 10^{-8} – 5 \times 10^{-7}$ M [45] with 15 – 30 % as free ions. Zn acts similarly to Fe with release in the rhizosphere due to decrease of pH are a result of proton pump [4]. In figure 49, the pattern is repeated (with exception of FeNP+His. 8 mg / L) that has been found in applications of Ca and Fe, higher mineral contractions at 5 cm with a lower concentration obtained at 30 cm, the region of tuberisation and development.

5.4. MONP application post-harvest

Pectobacterium spp. (PCA) characteristically produce large quantities of pectolytic enzymes [98] that are cell wall specific [99] which macerate plant tissue thus allowing infiltration and further tissue maceration [100]. PCA are one of a number of bacteria that cause a storage disease known as soft rot. Contamination from PCA occurs from soil during propagation and at harvest [100,101], plus pot-harvest handling, washing and packaging [2] through damage or poor storage.

A number of publications have reported [76,78,102] on the increase in Ca content of tubers increasing the resistance to tissue maceration via bacterial pathogens. Calcium enhances the structural integrity of cellular walls and membranes [77,98], therefore increased strength through via Ca application through fertiliser or as a post-harvest treatment offers an alternative to current chemical applications that are under scrutiny [102].

The application of CaNP is of great economic interest as a prevention of soft rot bacterial infection cause by Pectobacteria. The application of CaNP and FeNP controlled / retained the PCA from entering the tuber, thus reducing infection rate and potentially reduce crop loss while in storage.

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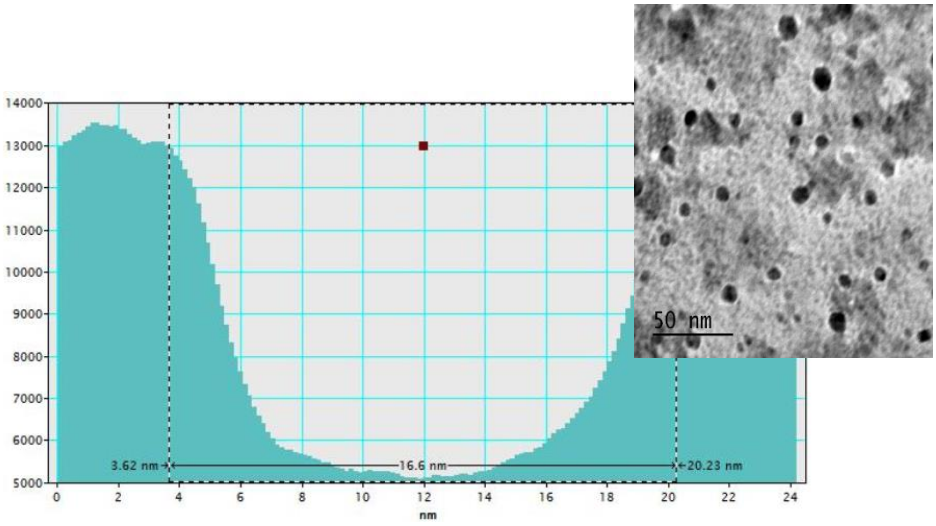
7. APPENDICES

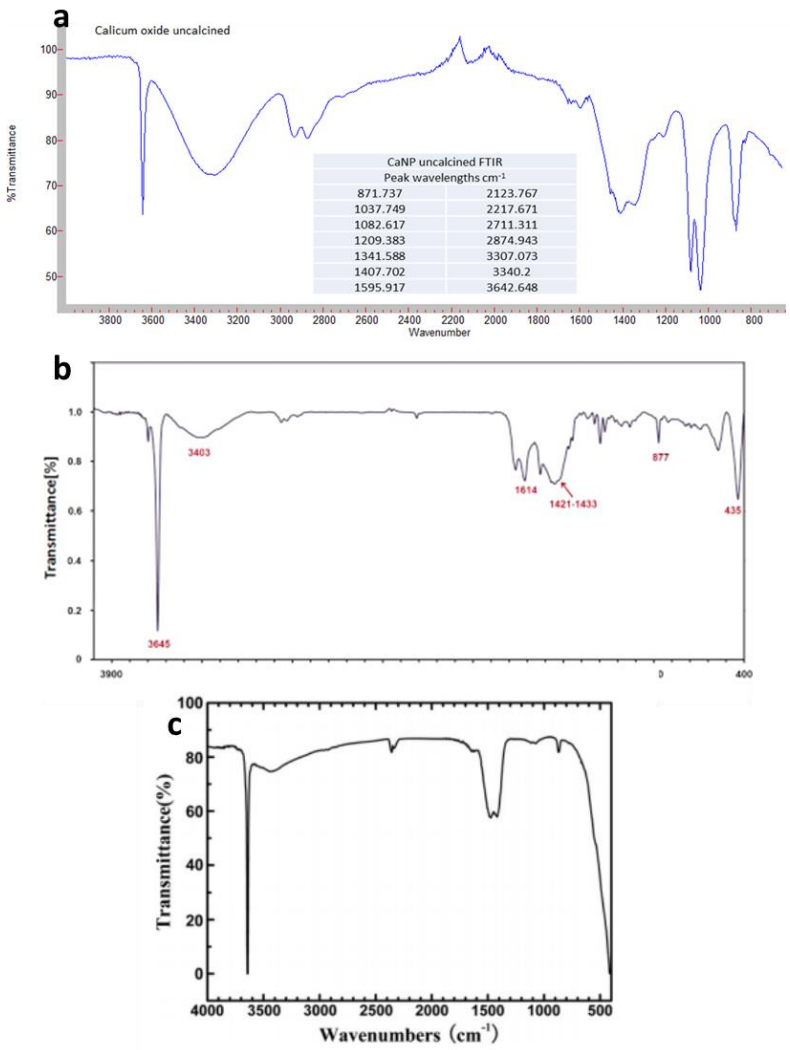
7.1. Abbreviations

AHDB	Agriculture and Horticulture Development Board
CaFeNP	Calcium ferrite nanoparticle ($CaFe_2O_4$)
CaFeNP+His	Calcium ferrite nanoparticle ($CaFe_2O_4$) coated with histidine 1:1, w/w
CaNP	Calcium oxide nanoparticle (CaO)
CaNP+His	Calcium oxide nanoparticle (CaO) coated with histidine 1:1, w/w
CFU	Colony forming unit
CSRC	Crop Storage Research Centre. AHDB Potatoes, Sutton Bridge, Lincolnshire
d.a.p	days after planting
DM%	Dry mass percentage
FeNP	Iron oxide nanoparticle (Fe_3O_4)
FeNP+His	Iron oxide nanoparticle (Fe_3O_4) coated with histidine 1:1, w/w
FTIR	Fourier-transform infrared spectroscopy
H_1	Alternative hypothesis
His	Histidine
HNS	Hydroponic nutrient solution
H_o	Null hypothesis
ICP-OES	inductively coupled plasma optical emission spectrometry
MONP	Metal oxide nanoparticle
MONP+His.	Metal oxide nanoparticle coated with histidine 1:1, w/w
NP	Nanoparticle
SDR	Spinning disc reactor
SEM	Scanning electron microscope
TF	Transfer factor
W.a.p.	Weeks after planting
XRD	X-ray powder diffraction
ZnNP	Zinc oxide nanoparticle (ZnO)
ZnNP+His	Zinc oxide nanoparticle (ZnO) coated with histidine 1:1, w/w

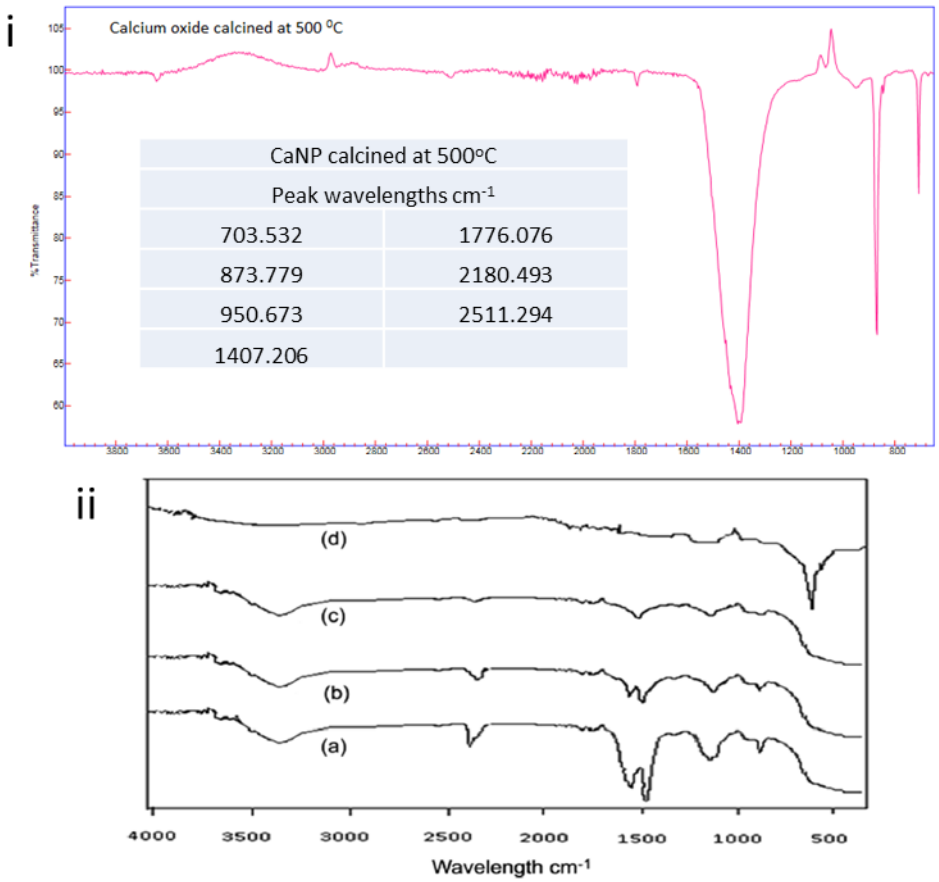
7.2. **Supporting data**



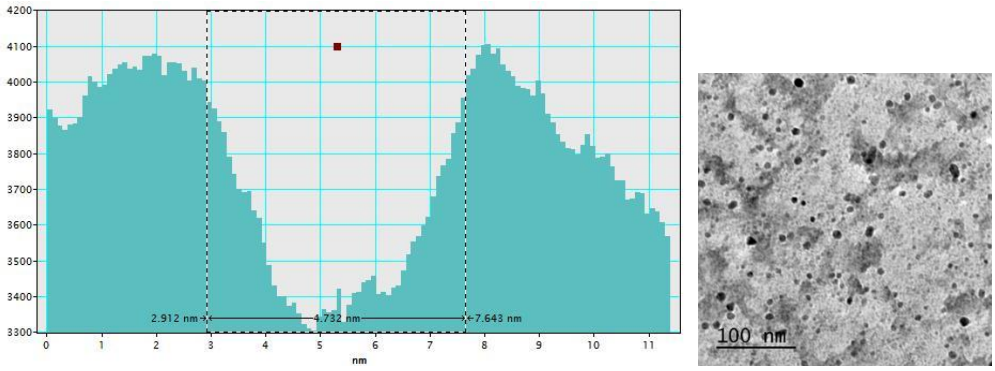
App. 1: Average dimensions of CaO nanoparticles (n=25). Inserted is the SEM of CaO, depicting the nanoparticles spherical shape. 3.62-20.18nm



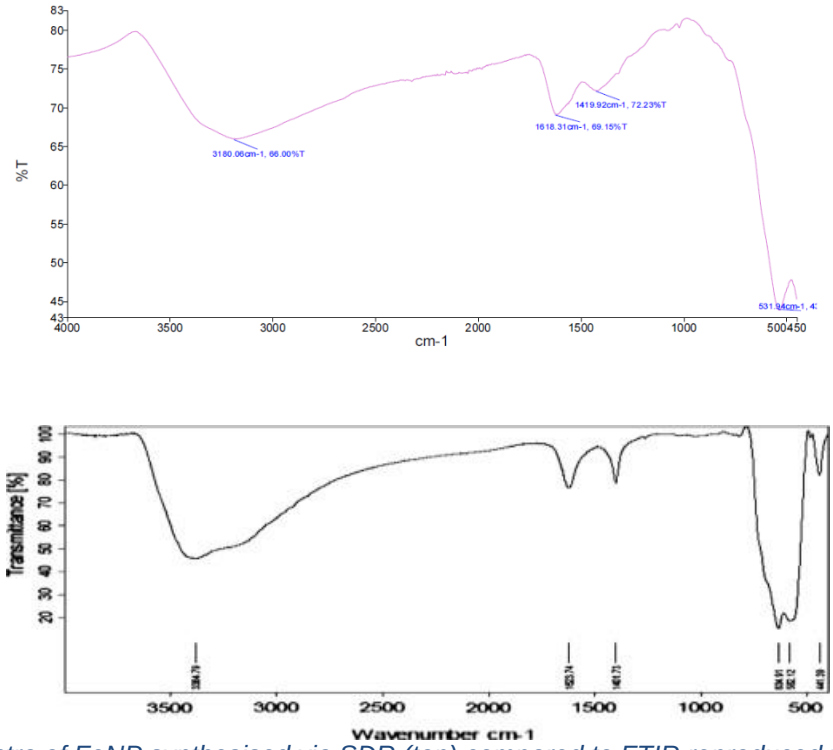
App. 2: Comparison of FTIR of uncalcined calcium oxide nanoparticles; a) CaNP synthesised from SDR, b) published by Darroudi et al., 2016 [118] and c) as published by Lui et al,. 2010 [119]



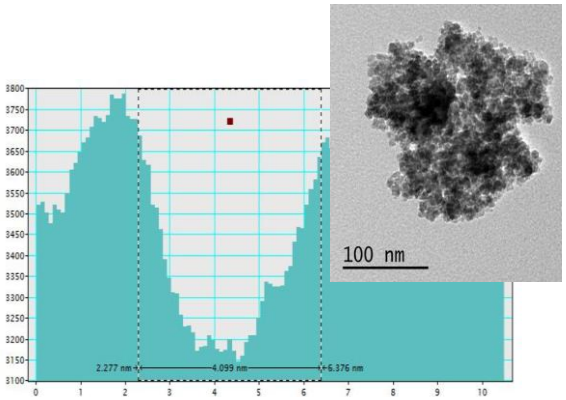
App. 3: Comparison of calcined CaO NP; i) CaO synthesised via SDR, and calcined at 500oC; ii) reproduced from FTIR published by Patel et al, 2009 [120], a) dried at room temperature, b) 350 oC, c) 550 oC and d) 900 oC.



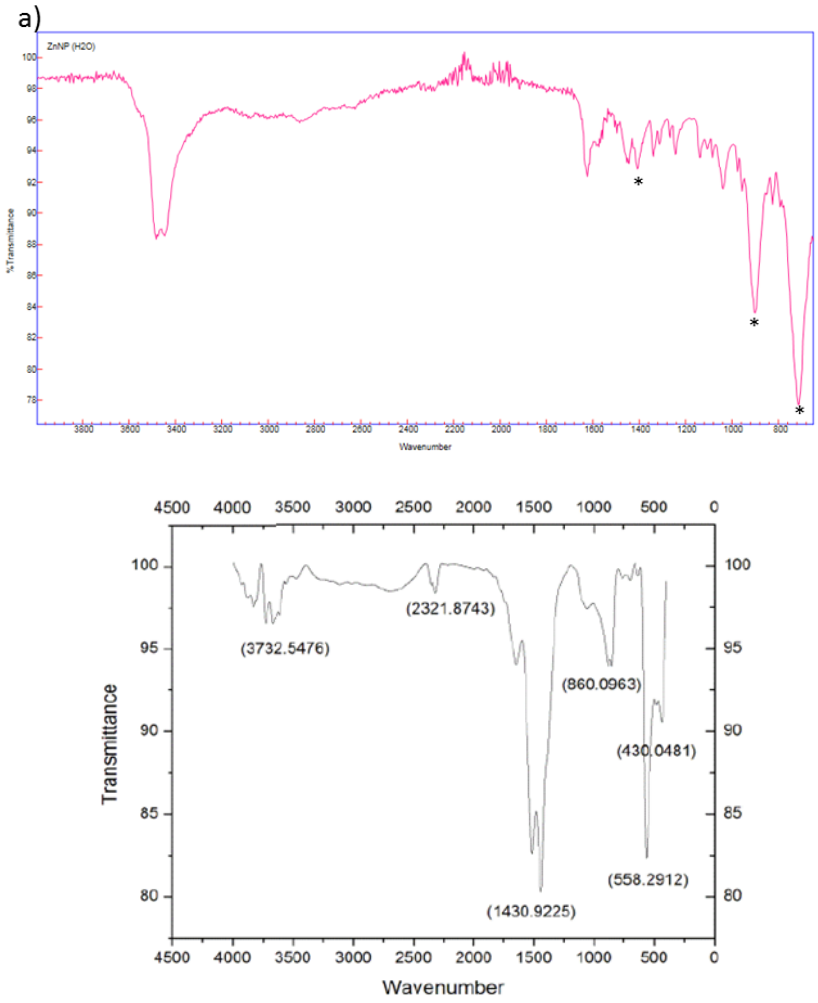
App. 4:FeNP synthesised via SDR (left) and size range (right) produced by SDR (TEM). NP ranging from 3 to 7.6 nm with an average of 4.732 nm



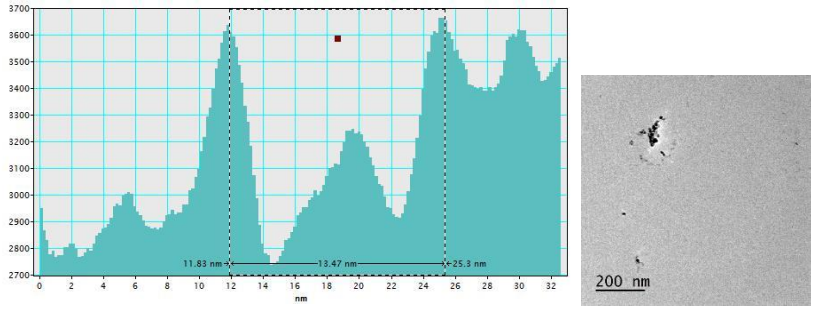
App. 5: FTIR spectra of FeNP synthesised via SDR (top) compared to FTIR reproduced from Khalil,2015 [121]



App. 6: Size range of ZnO nanoparticles synthesised from method adapted from Zak, et al. (2011)[105] with insert of TEM. Averaging 8.39 nm with a range of 7.03 to 15.41 nm



*App. 7 FTIR of a) ZnO nanoparticles synthesised with water as the precursor solvent. * indicates peaks matching zinc hydroxychloride [122]. Below, FTIR of ZnO synthesised from zinc chloride, reproduced from Rao and Rao (2015) [122].*



App. 8:Calcium ferrite nanoparticles (TEM) produced by sol-gel and thermal decomposition method

	Mean height (mm) at 6 weeks	*Percentage + or - in height against control*	*p value using ANOVA single factor*
Control	1198.73	N/A	N/A
FeNP+His 8 mg / L	1140.47	-4.86	0.2023
FeNP+His 12 mg / L	1234.40	2.98	0.2023
FeN+His 16 mg / L	1201.87	0.26	0.9246
CaNP+His 12 mg / L	1283.00	7.03	0.0501
CaNP+His 36 mg / L	1164.70	-2.84	0.2982
Ca.FeNP+His	1318.00	9.95	0.0210
ZnNP+His 8 mg / L	1300.80	8.51	0.0201
ZnNP+His 16 mg / L	1169.50	-2.44	0.7160

App. 9: Height (mm) of potato stems, percentage of height increase or decrease when compared to control six weeks after planting. P values attained from ANOVA single factor comparing control heights and treated plant stem heights at six weeks of growth. A p va

		Average height (mm) ± SD			
	Trial	*Week 3*	*Week 4*	*Week 5*	*p -value against control (for corresponding year) week 5*
Control 2014	H2014	524 ± 76.75	556.21 ± 92.95	578.08 ± 91.82	N/A
Control 2015	H2015	481 ± 152.14	613.79 ± 183.81	757.141 ± 91.82	N/A
CaNP+His 12 mg / L	H2015	35.57 ± 23.22	48.29 ± 22.26	66.00 ± 39.59	3.29×10^{-7}***
CaNP+His 32 mg / L	H2015	185.44 ± 117.08	284.44 ± 120.90	402.06 ± 119.63	1.15×10^{-5}***
FeNP+His 8 mg/L	H2015	477.2 ± 125.94	625.53 ± 96.33	791.40 ± 134.98	0.6308
FeNP+His 12 mg/L	H2015	444.64 ± 166.59	573.62 ± 159.06	736.69 ± 188.29	0.8058
FeNP+His 16 mg/L	H2015	390.35 ± 120.30	493.82 ± 108.83	654.41 ± 124.23	0.1783
Fe EDTA 8 mg/L	H2014	426.33 ± 133.54	441.06 ± 135.75	473.17 ± 150.57	0.0340*
ZnNP+His 8 mg / L	H2015	56.36 ± 41.40	60.91 ± 43.58	70.09 ± 41.69	1.81×10^{-9}***
ZnNP+His 16 mg / L	H2015	49.18 ± 28.03	51.73 ± 27.65	62.36 ± 36.93	1.40×10^{-9}***
His 8 mg/L	H2014	675.17 ± 977.77	728.75 ± 103.08	727.64 ± 129.68	3.27×10^{-3}**
His 20 mg/L	H2014	634.67 ± 149.31	677.33 ± 151.42	721.09 ± 161.12	0.0123*

App. 10: H2014 and H2015 growth data and statistical analysis

	Ca content (mg / L per gram of sample)	*p-value against control*	*p-value against CaNP+His. 12 mg / L*
Control 2015	346.86	N/A	N/A
CaNP+His 12 mg / L	480.04	0.0385	N/A
CaNP+His 32 mg / L	308.71	0.3621	$6.31x10^{-3}$**

App. 11: ICP analysis data of Ca content of tuber with statistical analysis. H2015

	Fe content (mg / L per gram of sample)	*p-value against control*	*p-value against FeNP+His 8 mg / L*	*p-value against FeNP+His 12 mg / L*
Control 2015	17.86	N/A	N/A	N/A
FeNP+His 8 mg/L	13.09	0.2781	N/A	N/A
FeNP+His 12 mg/L	27.79	0.5602	0.3868	N/A
FeNP+His 16 mg/L	7.24	0.0147*	0.0703	0.2295

App. 12: ICP analysis data of Fe content of tuber with statistical analysis. H2015

	Zn content (mg / L per gram of sample)	*p-value against control*	*p-value against ZnNP+His. 8 mg / L*
Control 2015	1.21	N/A	N/A
ZnNP+His 8 mg / L	5.05	$7.42x10^{-4}$***	N/A
ZnNP+His 16 mg / L	5.58	$2.19x10^{-7}$***	0.5816

App. 13: ICP analysis data of Fe content of tuber with statistical analysis.H2015

	Average height (mm) ± SD Week since planting				
	2	3	4	5	6
Control	325.01 ± 30.24	665.63 ± 29.91	919.95 ± 52.32	1138.87 ± 41.17	1198.73 ± 15.07
CaNP+His 12 mg / L	357.60 ± 88.61	742.40 ± 94.71	1005.60 ± 108.04	1184.40 ± 28.76	1315.20 ± 45.54
CaNP+His 36 mg / L	321.27 ± 39.74	689.07 ± 63.26	964.13 ± 40.13	1145.00 ± 23.84	1164.70 ± 46.81
Ca.FeNP+His	355.80 ± 20.54	648.93 ± 48.72	902.60 ± 53.79	1147.73 ± 31.34	1318.00 ± 46.10
FeNP+His 8 mg / L	336.95 ± 62.19	671.51 ± 59.27	944.53 ± 71.06	1142.85 ± 97.18	1027.04 ± 64.50
FeNP+His 12 mg / L	337.14 ± 52.16	683.63 ± 76.54	960.77 ± 32.12	1152.66 ± 37.08	1234.40 ± 41.58
FeNP+His 16 mg / L	331.48 ± 57.20	672.24 ± 49.95	986.09 ± 14.12	1192.34 ± 42.39	1350.60 ± 51.73
ZnNP+His 8 mg / L	335.19 ± 99.22	675.79 ± 69.33	963.80 ± 56.76	1147.75 ± 28.41	1130.72 ± 37.05
ZnNP+His 16 mg / L	339.40 ± 52.00	697.87 ± 64.21	990.67 ± 9.98	1187.13 ± 57.07	1169.50 ± 137.04

App. 14:Sax2015 growth rate over a 5-week period

	Average height (mm) ± SD Week since planting			
	3	4	5	6
Control (water only)	84.71 ± 66.85	442.89 ± 62.30	572.78 ± 32.26	637.83 ± 42.01
Chempak	207.75 ± 70.77	492.61 ± 101.48	622.61 ± 65.87	689.61 ± 56.76
CaNP+His 32 mg / L	116.06 ± 67.11	555.56 ± 76.58	700.39 ± 49.84	1175.22 ± 78.65
CaNP+His 64 mg / L	100.28 ± 62.62	550.78 ± 81.38	731.28 ± 40.55	806.06 ± 73.01
FeNP+His 16 mg / L	107.88 ± 69.85	496.94 ± 100.07	695.06 ± 38.64	736.17 ± 54.61
FeNP+His 32 mg / L	116.06 ± 54.54	510.44 ± 115.26	718.50 ± 85.48	819.33 ± 58.63
His 16 mg/L	104.73 ± 63.55	459.47 ± 106.76	676.88 ± 71.17	783.53 ± 60.05
His 32 mg/L	130.44 ± 48.11	499.67 ± 110.86	647.78 ± 95.42	776.56 ± 80.57
His 64 mg/l	127.56 ± 69.13	492.44 ± 101.49	636.33 ± 54.83	766.11 ± 41.50

App. 15: Sax2016 growth rate over a 4-week period

Sax2015	*p-value against control*	*Sax2016*	*p-value against control*	*p-value against Chempak*
CaNP+His 12 mg / L	0.2023	*Chempak*	$3.76x10^{-3}$**	N/A
CaNP+His 36 mg / L	0.2023	*CaNP+His 32 mg / L*	$6.27x10^{-7}$***	$2.00x10^{-3}$**
Ca.FeNP+His	0.9246	*CaNP+His 64 mg / L*	$6.77x10^{-10}$***	$6.17x10^{-6}$***
FeNP+His 8 mg / L	0.0501	*FeNP+His 16 mg / L*	$7.31x10^{-7}$***	0.0171*
FeNP+His 12 mg / L	0.2982	*FeNP+His 32 mg / L*	$2.13x10^{-12}$***	$9.46x10^{-8}$***
FeNP+His 16 mg / L	0.0210*	*His 16 mg/L*	$1.18x10^{-9}$***	$3.77x10^{-5}$***
ZnNP+His 8 mg / L	0.0201*	*His 32 mg/L*	$2.08x10^{-7}$***	$6.72x10^{-4}$***
ZnNP+His 16 mg / L	0.7160	*His 64 mg/l*	0.0219*	0.2764

App. 16:p-values of ANOVA single factor analysis of heights obtained at week 6 after planting.

		Percentage difference in height between week 3 and 6 (%)
Sax2015	Control	80.09
	CaNP+His 12 mg / L	77.16
	CaNP+His 36 mg / L	69.03
	Ca.FeNP+His	103.10
	FeNP+His 8 mg / L	52.95
	FeNP+His 12 mg / L	80.57
	FeN+His 16 mg / L	100.91
	ZnNP+His 8 mg / L	67.32
	ZnNP+His 16 mg / L	67.58
Sax2016	Control (water only)	653.00
	Chempak	231.94
	CaNP+His 32 mg / L	912.61
	CaNP+His 64 mg / L	703.82
	FeNP+His 16 mg / L	582.43
	FeNP+His 32 mg / L	605.96
	His 16 mg/L	648.12
	His 32 mg/L	495.35
	His 64 mg/l	500.58

App. 17: Percentage difference in height of stems between weeks 3 and 6 from trials Sax2015 and Sax2016, propagated in Erin multipurpose compost.

		Per plant	Total number harvested	Number of >30mm	Number of <30 mm	% >30mm	% <30mm
	Control	7.86	165	115	50	69.70	30.30
	CaNP+His 12 mg / L	7	182	129	53	70.88	29.12
	CaNP+His 36 mg / L	9.89	157	109	48	69.43	30.57
	Ca.FeNP+His	5.56	138	95	43	68.84	31.16
Sax2015	FeNP+His 8 mg / L	8.67	60	60	0	100.00	0.00
	FeNP+His 12 mg / L	7.48	61	61	0	100.00	0.00
	FeN+His 16 mg / L	6.57	63	47	16	74.60	25.40
	ZnNP+His 8 mg / L	6.57	89	60	29	67.42	32.58
	ZnNP+His 16 mg / L	6.67	50	48	2	96.00	4.00
	Control	11.50	207	140	67	67.63	32.37
	Chempak	11.17	201	112	90	55.72	44.28
	FeNP+His 16 mg/L	11.56	208	133	75	63.94	36.06
	FeNP+His 32 mg/L	9.17	165	114	51	69.09	30.91
Sax2016	CaNP+His 32 mg/L	9.72	175	128	47	73.14	26.86
	CaNP+His 64 mg/L	9.61	173	129	44	74.57	25.43
	His. 16 mg/L	9.67	174	130	44	74.71	25.29
	His 32 mg/L	10.78	194	133	61	68.56	31.44
	His. 64 mg/L	12.56	226	158	68	69.91	30.09

App. 18: Harvested number and segregation in to >30 mm and < 30 mm tubers

		Overall average weight (g)	*Average weight (g) > 30 mm*	*Average weight (g) < 30 mm*
Sax 2015	Control	39.81	55.01	4.83
	CaNP+His, 12 mg / L	49.09	63.82	5.82
	CaNP+His, 32 mg / L	35.52	50.36	4.81
	CaFeNP+His 24:12 mg / L	62.98	62.98	0.00
	FeNP+His 8 mg / L	41.71	55.87	7.23
	FeNP+His 12 mg / L	43.2	59.23	7.54
	FeNP+His 16 mg / L	40.46	55.37	6.73
	ZnNP+His 8 mg / L	49.67	49.67	0.00
	ZnNP+His 16 mg / L	55.43	55.43	0.00
Sax 2016	Control	29.81	41.51	5.36
	Chempak	29.79	48.21	6.55
	CaNP+His 32 mg/L	34.46	45.09	5.51
	CaNP+His 64 mg/L	33.89	43.93	4.45
	FeNP+His 16 mg/L	28.79	42.82	3.91
	FeNP+His 32 mg/L	31.45	43.34	4.88
	His. 16 mg/L	32.89	42.37	4.89
	His 32 mg/L	29.11	40.39	4.52
	His. 64 mg/L	29.03	39.04	5.77

App. 19: Harvested weights from trials Sax2015 and Sax2016, average tuber weight and average weight when segregated into >30mm and <30mm.

	Control	CaNP+His, 12 mg / L	CaNP+His, 32 mg / L	CaFeNP+His 24:12 mg / L	FeNP+His 8 mg / L	FeNP+His 12 mg / L	FeNP+His 16 mg / L	ZnNP+His 8 mg / L
Ca.								
Zn								

	Control	Chempak	CaNP+His 32 mg/L	CaNP+His 64 mg/L	FeNP+His 16 mg/L	FeNP+His 32 mg/L	His. 16 mg/L	His 32 mg/L
Chempak	0.9576	N/A	N/A	N/A	N/A	N/A	N/A	N/A
CaNP+His 32 mg/L	0.1238	0.1635	N/A	N/A	N/A	N/A	N/A	N/A
CaNP+His 64 mg/L	0.1704	0.2147	0.8700	N/A	N/A	N/A	N/A	N/A
FeNP+His 16 mg/L	0.7131	0.7862	0.0781	0.1088	N/A	N/A	N/A	N/A
FeNP+His 32 mg/L	0.5597	0.5842	0.3668	0.4578	0.3820	N/A	N/A	N/A
His. 16 mg/L	0.2655	0.3155	0.6310	0.7565	0.1700	0.6366	N/A	N/A
His 32 mg/L	0.7947	0.8631	0.0875	0.1215	0.9098	0.4238	0.1825	N/A
His. 64 mg/L	0.7725	0.8403	0.0816	0.1147	0.9325	0.4124	0.1825	0.9763

App. 22: p-values comparing average tuber weight of all tuber harvested in trial Sax2016

Trial (g).

	CaNP+His, 12 mg / L	CaNP+His, 32 mg / L	CAFeNP+His 24:12 mg / L	FeNP+His 8 mg / L	FeNP+His 12 mg / L	FeNP+His 16 mg / L	ZnNP+His 8 mg / L	ZnNP+His 16 mg / L
Control >30 mm	0.1328	0.2953	0.1572	0.8495	0.3658	0.9568	0.2838	0.9756
Control <30 mm	0.1001	0.4134	N/A	0.0117	0.0032	0.0239	N/A	N/A

App. 21: Statistical analysis of tuber weight average when segregated into size. Trial Sax2015

	Chempak	FeNP+His 16 mg/L	FeNP+His 32 mg/L	CaNP+His 32 mg/L	CaNP+His 64 mg/L	His. 16 mg/L	His 32 mg/L	His. 64 mg/L
Control >30 mm	0.0784	0.6858	0.5626	0.3020	0.4724	0.7768	0.7123	0.415074
Chempak >30 mm	N/A	0.1969	0.2450	0.4791	0.3211	0.1451	0.0490	0.018389

Control <30 mm	0.0585	0.0020	0.4128	0.7983	0.1542	0.4361	0.114179	0.852794
Chempak <30 mm	N/A	$3.38x10^{-6}$	0.0150	0.1361	0.0051	0.0219	0.001276	0.689115

App. 23: Statistical analysis of tuber weight average when segregated into size. Trial Sax2015

Trial	*Treatment*	*DM % ± SD*
Sax2015	Control	36.67 ± 3.33
	CaNP+His 12 mg / L	35.24 ± 2.45
	CaNP+His 36 mg / L	36.29 ± 3.60
	Ca.FeNP+His	33.44 ± 2.14
	FeNP+His 8 mg / L	35.69 ± 3.50
	FeNP+His 12 mg / L	32.67 ± 4.24
	FeN+His 16 mg / L	35.03 ± 2.32
	ZnNP+His 8 mg / L	33.39 ± 2.85
	ZnNP+His 16 mg / L	34.17 ± 2.01
Sax2016	Control	39.59 ± 3.87
	Chempak	38.08 ± 3.19
	FeNP+His 16 mg/L	38.95 ± 2.53
	FeNP+His 32 mg/L	37.87 ± 2.79
	CaNP+His 32 mg/L	35.61 ± 3.08
	CaNP+His 64 mg/L	39.67 ± 2.64
	His 16 mg/L	36.69 ± 4.04
	His 32 mg/L	37.72 ± 2.71
	His 64 mg/L	49.92 ± 5.45

App. 24: Percentage of dry matter (DM%) Sax2015 and Sax2016

p-values DM% Sax2015	*Control*	*CaNP+His, 12 mg / L*	*CaNP+His, 32 mg / L*	*FeNP+His 8 mg / L*	*FeNP+His 12 mg / L*	*ZnNP+His 8 mg / L*
CaNP+His 12 mg / L	0.2595	N/A	N/A	N/A	N/A	N/A
CaNP+His 32 mg / L	0.7830	0.4823	N/A	N/A	N/A	N/A
Ca.FeNP+His	0.0126	0.1155	0.0582	N/A	0.6109	N/A
FeNP+His 8 mg / L	0.3607	N/A	N/A	N/A	N/A	N/A
FeNP+His 12 mg / L	$1.54x10^{-3}$	N/A	N/A	0.0159	N/A	N/A
FeN+His 16 mg / L	0.0725	N/A	N/A	0.4776	0.0308	N/A
ZnNP+His 8 mg / L	0.0467	N/A	N/A	N/A	N/A	N/A
ZnNP+His 16 mg / L	0.4776	N/A	N/A	N/A	N/A	0.4545

App. 25: p-value between DM % Sax2015

p-vlaues DM% Sax2016	*Control*	*Chempak*	*CaNP+His 64 mg/L*	*FeNP+His 16 mg/L*	*FeNP+His 32 mg/L*	*His. 16 mg/L*	*His 32 mg/L*
Chempak	0.1867	N/A	N/A	N/A	N/A	N/A	N/A
Ca*NP+His 32 mg/L*	$9.24x10^{-4}$	0.0175	N/A	N/A	N/A	N/A	N/A

CaNP+His 64 mg/L	0.9355	0.0937	N/A	N/A	N/A	N/A	N/A
FeNP+His 16 mg/L	0.5427	0.3442	N/A	N/A	N/A	N/A	N/A
FeNP+His 32 mg/L	0.1150	0.8245	N/A	0.2055	0.0269	N/A	N/A
His. 16 mg/L	0.0264	0.2377	N/A	0.0411	N/A	N/A	N/A
His 32 mg/L	0.0857	0.7072	N/A	N/A	0.8705	0.3501	N/A
His. 64 mg/L	$1.85x10^{-8}$	$1.76x10^{-10}$	$2.06x10^{-9}$	N/A	N/A	$7.13x10^{-11}$	2.91x10-3

App. 26: p-value between DM % Sax2016

DM% comparison	*p-value*
FeNP+His. 16 mg / L	7.32E-06
CaNP+His. 32 mg / L	0.6085
Control with Chempak Sax2016	0.1737

App. 27: Comparison of DM % between same applications between trials Sax2015 and Sax2016

	Ca content (mg / L per gram)		
	Whole tuber	Skin	Tuber flesh
Control	115.82	121.95	113.77
CaNP+His. 12 mg / L	145.60	191.99	130.14
CaNP+His. 32 mg / L	49.58	84.39	49.58
CaFeNP+His. (24:12 mg / L)	221.45	393.58	164.08

App. 28: Concentration of Ca from tubers harvested from trial Sax2015, treated with MONP+His.

p-values Sax2015 Whole tuber	*CaNP+His. 12 mg / L*	*CaNP+His. 32 mg / L*	*CaFeNP+His. (24:12 mg / L)*
Control	0.0192	$2.62x10^{-16}$	$4.3x10^{-9}$
CaNP+His. 12 mg / L	*N/A*	$1.03x10^{-11}$	$2.97x10^{-4}$
CaNP+His. 32 mg / L	*N/A*	*N/A*	$2.47x10^{-9}$

App. 29: Statistical p-values for the comparison of whole tuber Ca content of Sax2015 tubers

p-values Sax2015 Skin	*CaNP+His. 12 mg / L*	*CaNP+His. 32 mg / L*	*CaFeNP+His. (24:12 mg / L)*
Control	0.4046	0.0461	$2.57x10^{-6}$
CaNP+His. 12 mg / L	*N/A*	0.0557 0.0278 *	$6.54x10^{-5}$
CaNP+His. 32 mg / L	*N/A*	*N/A*	$4.28x10^{-4}$

p-values Sax2015 Tubers	*CaNP+His. 12 mg / L*	*CaNP+His. 32 mg / L*	*CaFeNP+His. (24:12 mg / L)*
Control	0.0127	$1.95x10^{-19}$	$1.16x10^{-9}$
CaNP+His. 12 mg / L	*N/A*	$8.92x10^{-15}$	$1.48x10^{-3}$
CaNP+His. 32 mg / L	*N/A*	*N/A*	$5.24x10^{-17}$

*App. 30: Statistical p-values for the comparison of skin and tuber content of Ca from trial Sax2015. * p-value of one-way t-test.*

	Fe content (mg / L per gram)		
	Whole tuber	Skin	Tuber
Control	26.53	29.17	25.65
FeNP+His. 8 mg / L	23.56	27.91	22.11
FeNP+His. 12 mg / L	31.39	76.06	16.50
FeNP+His. 16 mg / L	36.11	48.82	31.87
CaFeNP+His. (24:12 mg / L)	15.72	12.48	1.71

App. 31: Concentration of Fe from tubers harvested from trial Sax2015, treated with MONP+His.

p-values Sax2015 Whole tuber	*FeNP+His. 8 mg / L*	*FeNP+His. 12 mg / L*	*FeNP+His. 16 mg / L*	*CaFeNP+His. (24:12 mg / L)*
Control	0.3686	0.5746	$5.60X10^{-3}$	$4.11x10^{-18}$
FeNP+His. 8 mg / L	*N/A*	0.320828	0.001641	$1.28x10^{-22}$
FeNP+His. 12 mg / L	*N/A*	*N/A*	0.0559 0.0280 *	$4.31x10^{-6}$
FeNP+His. 16 mg / L	*N/A*	*N/A*	*N/A*	$2.93x10^{-7}$

*App. 32: Statistical p-values for the comparison of whole tuber Fe content of Sax2015 tubers. * p-value of one-way t-test.*

p-values Sax2015 Skin	*FeNP+His. 8 mg / L*	*FeNP+His. 12 mg / L*	*FeNP+His. 16 mg / L*	*CaFeNP+His. (24:12 mg / L)*
Control	0.8716	0.0845	0.1471	0.1812
FeNP+His. 8 mg / L	*N/A*	0.0656 0.0328*	0.0648 0.0324*	$7.00x10^{-4}$
FeNP+His. 12 mg / L	*N/A*	*N/A*	0.3302	0.1171
FeNP+His. 16 mg / L	*N/A*	*N/A*	*N/A*	0.0430

p-values Sax2015 Tuber	FeNP+His. 8 mg / L	FeNP+His. 12 mg / L	FeNP+His. 16 mg / L	CaFeNP+His. (24:12 mg / L)
Control	0.0198	$7.88x10^{-7}$	0.0935	7.1710^{-24}
FeNP+His. 8 mg / L	*N/A*	$7.43x10^{-4}$	$7.96x10^{-3}$	$2.58x10^{-25}$
FeNP+His. 12 mg / L	*N/A*	*N/A*	$6.95x10^{-5}$	$1.72x10^{-11}$
FeNP+His. 16 mg / L	*N/A*	*N/A*	*N/A*	$1.09x10^{-7}$

*App. 33: Statistical p-values for the comparison of skin and tuber content of Fe from trial Sax2015. * p-value of one-way t-test.*

	Zn content (mg / L per gram)		
	Whole tuber	Skin	Tuber
Control	7.91	9.50	7.38
ZnNP+His. 8 mg / L	145.08	243.38	112.31
ZnNP+His. 16 mg / L	36.09	61.38	25.25

App. 34: Concentration of Zn from tubers harvested from trial Sax2015, treated with MONP+His.

p-values Sax2015 Whole tuber	*ZnNP+His. 8 mg / L*	*ZnNP+His. 16 mg / L*
Control	$8.65x10^{-8}$	$2.19x10^{-20}$
ZnNP+His. 8 mg / L	*N/A*	$9.30x10^{-3}$

App. 35: Statistical p-values for the comparison of whole tuber Zn content of Sax2015 tubers.

p-values Sax2015 Skin	*ZnNP+His. 8 mg / L*	*ZnNP+His. 16 mg / L*
Control	$2.84x10^{-5}$	$7.65x10^{-12}$
ZnNP+His. 8 mg / L	*N/A*	0.0251

p-values Sax2015 Tuber	ZnNP+His. 8 mg / L	ZnNP+His. 16 mg / L
Control	$1.98x10^{-4}$	$8.60x10^{-19}$
ZnNP+His. 8 mg / L	*N/A*	0.0720 0.0232*

*App. 36: Statistical p-values for the comparison of skin and tuber content of Fe from trial Sax2015. * p-value of one-way t-test.*

	Ca content (mg / L per gram)			
	Whole tuber	Skin / cortex	Parenchyma / vascular ring	Perimedulla / medulla
Control	242.41	58.84	11.58	14.79
Chempak	267.51	66.10	11.38	16.09
FeNP+His 16 mg/L	N/A	100.98	14.75	28.88
FeNP+His 32 mg/L	N/A	76.27	20.51	20.27
CaNP+His 32 mg/L	290.93	77.57	15.17	28.52
CaNP+His 64 mg/L	243.88	65.99	16.98	19.66
His 16 mg/L	N/A	73.35	14.46	22.21
His 32 mg/L	250.99	87.17	10.76	18.73
His 64 mg/L	140.77	45.91	16.46	15.00

App. 37: Concentration of Ca from tubers (and constituent parts) harvested from trial Sax2016, treated with MONP+His.

p-value of whole tuber	*Control*	*Chempak*	*CaNP+His 32 mg/L*	*CaNP+His 64 mg/L*	*His 32 mg/L*
Chempak	$1.06x10^{-5}$	N/A	N/A	N/A	N/A
CaNP+His 32 mg/L	0.0203	0.2572	N/A	N/A	N/A
CaNP+His 64 mg/L	0.9323	0.1820	0.0790 0.0395*	N/A	N/A
His 32 mg/L	0.6487	0.3874	0.1490	N/A	N/A
His 64 mg/L	$4.19x10^{-24}$	$8.31x10^{-26}$	N/A	$3.74x10^{-7}$	$5.05x10^{-7}$

*App. 38: p-value of Ca content of whole tuber analysis from Sax2016 trial. * p-value of one-way t-test*

p-values of Ca content Sax2016	*Skin / cortex*	*Parenchyma / vascular ring*	*Perimedulla / medulla*
Chempak	$2.41x10^{-9}$	0.0705	$1.75x10^{-6}$
CaNP+His 32 mg/L	$1.12x10^{-13}$	$1.75x10^{-8}$	$1.62x10^{-13}$

CaNP+His 64 mg/L	$2.24x10^{-10}$	$8.48x10^{-10}$	$1.49x10^{-10}$
His 32 mg/L	$1.20x10^{-13}$	$4.77x10^{-4}$	$4.41x10^{-10}$
His 64 mg/L	$1.24x10^{-11}$	$2.84x10^{-9}$	0.1253

App. 39: Statistical p-values for comparison of Ca content of tuber constituents from trial Sax2016.

	Fe content (mg / L per gram)			
	Whole tuber	Skin / cortex	Parenchyma / vascular ring	Perimedulla / medulla
Control	165.24	170.55	82.67	88.22
Chempak	182.21	167.03	71.93	103.11
CaNP+His 32 mg/L	142.69	224.84	89.99	102.78
CaNP+His 64 mg/L	136.13	156.36	96.38	100.90
FeNP+His 16 mg/L	194.70	171.92	75.94	93.93
FeNP+His 32 mg/L	150.48	148.96	74.62	101.91
His 16 mg/L	136.12	269.09	81.96	104.49
His 32 mg/L	127.99	236.12	93.10	102.29
His 64 mg/L	84.78	141.34	99.45	93.73

App. 40: Concentration of Fe from tubers (and constituent parts) harvested from trial Sax2016, treated with MONP+His.

	Control	*Chempak*	*FeNP+His 16 mg/L*	*FeNP+His 32 mg/L*	*CaNP+His 32 mg/L*	*CaNP+His 64 mg/L*	*His 16 mg/L*	*His 32 mg/L*
Chempak	0.01256	N/A	N/A	N/A	N/A	N/A	N/A	N/A
FeNP+His 16 mg/L	0.0011	0.1695	N/A	N/A	N/A	N/A	N/A	N/A

FeNP+His 32 mg/L	0.0886	$1.70x10^{-3}$	$2.12x10^{-4}$	N/A	N/A	N/A	N/A	N/A
CaNP+His 32 mg/L	$1.62x10^{-5}$	$1.45x10^{-7}$	N/A	0.3304	N/A	N/A	N/A	N/A
CaNP+His 64 mg/L	$8.35x10^{-7}$	$6.85x10^{-11}$	N/A	N/A	0.1886	N/A	N/A	N/A
His 16 mg/L	0.0041	$2.40x10^{-5}$	$6.81x10^{-7}$	N/A	N/A	N/A	N/A	N/A
His 32 mg/L	$2.21x10^{-5}$	$2.57x10^{-8}$	N/A	0.0148	0.0655	N/A	0.2766	x
His 64 mg/L	$1.94x10^{-21}$	$1.65x10^{-23}$	N/A	N/A	N/A	$1.09x10^{-20}$	$9.36x10^{-12}$	$1.54x10^{-11}$

App. 41: p-value of Ca content of whole tuber analysis from Sax2016 trial.

Skin / cortex	*Control*	*Chempak*	*FeNP+His 16 mg/L*	*FeNP+His 32 mg/L*
Chempak	0.0562	N/A	N/A	N/A
FeNP+His 16 mg/L	0.5612	0.0141	N/A	N/A
FeNP+His 32 mg/L	$7.49x10^{-9}$	$1.53x10^{-11}$	$4.90x10^{-9}$	N/A
His 16 mg/L	$1.20x10^{-15}$	$3.65x10^{-17}$	$0.77x10^{-15}$	N/A
His 32 mg/L	$1.49x10^{-14}$	$2.80x10^{-17}$	N/A	$2.80x10^{-18}$

App. 42: Statistical p-values for comparison of Fe content of tuber skin / cortex from trial Sax2016.

Parenchyma / vascular ring	*Control*	*Chempak*	*FeNP+His 16 mg/L*	*FeNP+His 32 mg/L*
Chempak	$4.09x\ 10^{-12}$	N/A	N/A	N/A
FeNP+His 16 mg/L	$8.58x10^{-7}$	$2.16x10^{-4}$	N/A	N/A
FeNP+His 32 mg/L	$2.22x10^{-9}$	$5.49x10^{-4}$	$1.02x10^{-6}$	N/A
His 16 mg/L	0.0690	$1.54x10^{-13}$	0.1542	N/A
His 32 mg/L	$3.20x10^{-14}$	$2.29x10^{-14}$	N/A	$6.57x10^{-13}$

App. 43: Statistical p-values for comparison of Fe content of tuber parenchyma / vascular ring from trial Sax2016.

Perimedulla / medulla	*Control*	*Chempak*	*FeNP+His 16 mg/L*	*FeNP+His 32 mg/L*
Chempak	$8.95x10^{-10}$	N/A	N/A	N/A
FeNP+His 16 mg/L	$1.43x10^{-7}$	$1.09x10^{-6}$	N/A	N/A
FeNP+His 32 mg/L	$3.93x10^{-9}$	0.4001	$7.10x10^{-6}$	N/A

His 16 mg/L	$9.49x10^{-5}$	0.2207	$1.97x10^{-10}$	N/A
His 32 mg/L	$2.33x10^{-13}$	0.4676	N/A	0.7412

App. 44: Statistical p-values for comparison of Fe content of tuber perimedulla / medulla from trial Sax2016.

	Ca conc. at 5 cm (mg/L)	*Ca conc. at 30 cm (mg / L)*	*p-value between 5 and 30 cm*
Compost before application	7717.24		N/A
Control	7970.50	6074.60	0.0763
CaNP+His. 12 mg / L	7126.11	5213.85	$9.65x10^{-5}$
CaNP+His. 32 mg / L	9771.83	4781.88	$3.90x10^{-5}$
CaFeNP+His. (24:12) mg / L	7078.92	5638.41	0.0715
	Fe conc. at 5 cm (mg/L)	*Fe conc. at 30 cm (mg / L)*	p-value between 5 and 30 cm
Compost before application	267.45		N/A
Control	262.45	243.31	$4.41x10^{-3}$
FeNP+His. 8 mg / L	180.85	214.42	$5.51x10^{-4}$
FeNP+His. 12 mg / L	242.07	235.89	0.3996
FeNP+His. 16 mg / L	220.30	211.18	0.2347
CaFeNP+His. (24:12) mg / L	234.62	236.87	0.8629
	Zn conc. at 5 cm (mg/L)	*Zn conc. at 30 cm (mg / L)*	p-value between 5 and 30 cm
Compost before application	42.52		N/A
Control	37.01	17.54	0.0139
ZnNP+His. 8 mg / L	259.18	100.14	$3.88x10^{-8}$
ZnNP+is. 16 mg / L	321.34	36.45	$3.73x10^{-5}$

App. 45: ICP results from the retention of MONP from ICP of compost form trial Sax2015 with p-value comparing mineral concentrations between depth 5 and 30 cm.

CaNP	*5 cm*	*30 cm*
Compost before app.	0.8140	0.2252
CaNP+His. 12 mg / L	0.2126	0.2300
CaNP+His. 32 mg / L	0.0537	0.1035

CaFeNP+His. (24:12) mg / L	0.3583	0.5088

App. 46: p-value of CaNP retention in compost Sax2015 against control.

FeNP	*5 cm*	*30 cm*
FeNP+His. 8 mg / L	1.86×10^{-6}	8.81×10^{-5}
FeNP+His. 12 mg / L	0.0246	0.1159
FeNP+His. 16 mg / L	2.64×10^{-4}	2.88×10^{-4}
CaFeNP+His. (24:12) mg / L	0.0684	0.0630

App. 47: p-value of FeNP retention in compost Sax2015 against control.

ZnNP	*5 cm*	*30 cm*
Compost before app.	0.5963	7.45×10^{-3}
ZnNP+His. 8 mg / L	1.92×10^{-9}	4.18×10^{-10}
ZnNP+is. 16 mg / L	3.52×10^{-5}	0.0545

App. 48: p-value of ZnNP retention in compost Sax2015 against control.

	Average height (mm)					*p-value of heights at week 7 against control*
	Week 3	Week 4	Week 5	Week 6	Week 7	
Control	204.53	336.33	365.63	439	478	N/A
Drench	226.27	342.27	413.73	440.9	482	0.7957
Drench + 5-week app	223.47	338.80	417.6	485.27	546.2	1.09×10^{-4}

App. 49: Growth rates of stems from trial FieldRep2016 and p-values of height at 7 weeks against control.

Treatment	*Plot wt (kg)*	*Tuber numbers 10-20mm*	*Tuber wt (kg) 10-20mm*	*Tuber numbers 20-40mm*	*Tuber wt (kg) 20-40mm*	*Tuber numbers 40-65mm*	*Tuber wt (kg) 40-65 mm*	*Tuber numbers 65 +*	*Tuber wt (kg) 65 +*
1	2.78	0.00	0.00	24.25	0.97	17.50	1.75	0.00	0.00
2	3.14	0.00	0.00	24.00	0.94	21.00	2.12	0.00	0.00
3	2.99	0.00	0.00	24.25	0.99	19.00	1.87	0.00	0.00
4	3.08	0.00	0.00	24.00	0.94	20.50	2.00	0.00	0.00

App. 50: Harvest data collated by Branston Plc for Field2015

		Treatment 1 (control)	*Treatment 2*	*Treatment 3*	*Treatment 4*
DM% ± SD	*Midway*	39.94 ± 2.69	41.06 ± 3.52	38.82 ± 3.05	40.76 ± 3.45
	At harvest	34.36 ± 2.97	36.97 ± 3.41	37.48 ± 4.73	37.04 ± 3.99
Fe content (mg / L)	*Midway*	20.07	9.00	26.03	20.58

At harvest	66.17	81.93	67.58	55.57

App. 51: Comparison of DM% and Fe content (mg / L per gram) midway (12th week after planting) and at harvest (21.5 weeks).

	p-values of DM% midway			*p-value of DM% at harvest*		
	Against control (T1)	Against T2	Against T3	Against control (T1)	Against T2	Against T3
T2	0.1578	N/A	N/A	$1.79x10^{-3}$	N/A	N/A
T3	0.1229	$8.37x10^{-3}$	N/A	0.0250	0.6276	N/A
T4	0.2940	0.7298	0.0200	$3.47x10^{-3}$	0.9458	0.6894

App. 52: Statistical comparison of DM% midway through trial and at harvest. Field2015.

	p-values between DM% midway and at harvest
T1	$6.41x10^{-11}$
T2	$1.38x10^{-5}$
T3	0.1814
T4	$1.73x10^{-4}$

App. 53: p-values of the comparison of DM% with in treatment midway and at harvest

	p-values of Fe content midway			*p-value of Fe content at harvest*		
	Against control (T1)	Against T2	Against T3	Against control (T1)	Against T2	Against T3
T2	0.0369	N/A	N/A	0.1787	N/A	N/A
T3	0.1263	$7.88x10^{-6}$	N/A	0.8872	0.1728	N/A
T4	0.8763	$1.25x10^{-3}$	0.0541	0.2300	$6.16x10^{-3}$	0.0952

App. 54: p-values of Fe content midway through trial (week 12) and at harvest (week 21).

	p-values between Fe content midway and at harvest
T1	$5.75x10^{-7}$
T2	$1.34x10^{-4}$
T3	$1.68x10^{-5}$
T4	$5.30x10^{-10}$

App. 55: p-values comparing the Fe content of tubers midway (week 12) and at harvest (week 21) with in treatments.

Against control (T1)	*Number of tuber (p-value)*	*weight of tubers (p-vaules*
T2	>0.25	>0.25
T3	>0.25	0.0005

T4	0.005	>0.25

App. 56:chi-squared analysis against Treatment 1 (control), 20-40 mm and 40-65 mm distribution in number and weight (kg).

Against control (T1)	*Total no. of tubers*	*Total weight*	*No. of tubers 20- 40 mm*	*Wt. of tubers 20-40 mm*	*No. of tubers 40-65 mm*	*Wt. of tubers 40-65 mm*
T2	0.8443	0.9363	1.0000	0.8575	0.8623	0.8133
T3	0.8362	0.5556	0.8356	0.7383	0.9442	0.8646
T4	0.7563	0.9344	0.2012	0.2899	0.4659	0.6169

App. 57: p-values from ANOVA one-way statistical analysis of tuber numbers and weights from Field2015.

	p-values of Fe content midway			*p-value of Fe content at harvest*		
	Against control (T1)	Against T2	Against T3	Against control (T1)	Against T2	Against T3
T2	0.7800	N/A	N/A	0.6942	N/A	N/A
T3	0.9281	0.6818	N/A	0.1480	0.2780	N/A
T4	0.2127	0.1071	0.2074	0.9780	0.6938	0.0808

App. 58: p-value of soil samples before and after trial, Field2015.

	p-values between Fe content midway and at harvest
T1	3.16×10^{-3}
T2	0.0410
T3	3.70×10^{-3}
T4	1.89×10^{-3}

App. 59: p-value from the comparison between Fe content of soil before and after trial, Field2015

Variety and treatment	*Site 1*	*Site 2*
Maris piper - Control	39.49 ± 3.62	36.86 ± 3.50
Maris piper - Treated	39.91 ± 3.31	38.87 ± 3.79
Inca bella - Control	38.38 ± 2.19	39.13 ± 1.94
Inca bella Treated	39.06 ± 2.01	39.71 ± 2.18

App. 60: DM % ± SD obtained from Field2016 trial comparing the effect of variety, treatment and location.

	Site 1	*Site 2*
Maris piper	0.5925	0.0157
Inca bella	0.1547	0.2095

App. 61: Field2016, p-values of DM% of treatment against control.

		Fe content (mg / L per gram)	
		Control	Treatment
Inca Bella	Site A	54.45	55.32
	Site B	48.07	53.77
Maris piper	Site A	41.38	69.76
	Site B	59.38	68.24
Inca Bella	Overall av.	51.26	54.55
Maris piper	Overall av.	50.38	69.00

App. 62: Fe content of tubers segregated into different locations to compare uptake of Fe in different soils and varieties of potato, Field2016

		p-value
Inca Bella	Site A	0.8567
	Site B	0.1155
Maris piper	Site A	0.0167
	Site B	0.3002
Inca Bella	Overall av.	0.2766
Maris piper	Overall av.	0.0108

App. 63: Fe content of tubers across both locations. Field2016

		Av. 59Fe (MBq)	*Av .Fe conc (mg / L per gram)*
FeNP+His	Soil	398.00	96.68
	Tuber	33.00	0.98
	Stem lower	13.00	12.99
	Stem mid	3.00	1.22
	Stem top	16.00	2.55
Fe-EDTA	Soil	24.29	4.52
	Tuber	0.22	0.00
	Stem lower	21.67	4.70
	Stem mid	10.67	0.92
	Stem top	5.83	0.62

App. 64: Data collected from trial 59Fe initially read in MBq then converted to Fe content via calibration graph.

	Fe-EDTA against ^{59}FeNP+His
	p-*value*
Soil	1.14×10^{-9}
Tuber	1.17×10^{-8}

Stem lower	1.61×10^{-8}
Stem mid	9.12×10^{-5}
Stem top	7.94×10^{-5}

App. 65:p-values of Fe content (mg / L) comparing 59Fe-EDTA to 59FeNP+His data.

	p-values of swab data				*p-values of peel data*			
	2	3	1a	2a	2	3	1a	2a
1	6.87×10^{-4}	0.4918	1.72×10^{-5}	N/A	0.1467	0.0801	0.2887	N/A
2	N/A	6.88×10^{-4}	N/A	N/A	N/A	0.8337	N/A	N/A
2a	4.30×10^{-5}	N/A	1.81×10^{-3}	N/A	0.6800	N/A	0.4039	N/A
3a	N/A	0.0248	0.5754	2.33×10^{-3}	N/A	0.2888	0.8677	0.5300

App. 66: p-values to compare the effect of MONP and PW