

Exploration of shrimp shell chitin as an agricultural biostimulant and its effects on soil microbial respiration

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Aims and Objectives

To investigate the effect of chitin ((Poly-(1→4)-β-N-acetyl-D-glucosamine) biostimulant addition on soil microorganism metabolism in agricultural soils by:

- The addition of multiple shrimp shell concentrations and particle size fractions to agricultural soil mesocosms and measure greenhouse gas production.
- Use of enzyme assays to determine substrate type utilisation

To understand how stoichiometry effects biostimulant addition and impacts on soil quality..

Method

Soil samples were collected to 15cm depth along a transect from a Ploughed field and particles homogenised to 4mm. Mesocosms were created containing 1, 5 and 10% of shrimp shell chitin (w/w), in 63 to 2000µm particle size fractions (see figure 1). The soils were maintained at 50% field capacity and 25°C.

A Greenhouse Gas Analyser (Los Gatos, USA) measured CO₂ and CH₄ pre- and post experiment for microbial respiration. H₂O, soil organic matter content and pH were monitored throughout. Assays of glycosidase, chitinase, phosphatase and phenol oxidase reflected soil metabolic capacity. Statistical analysis was performed with R studio using a one-way ANOVA or Kruskal – Wallis test.

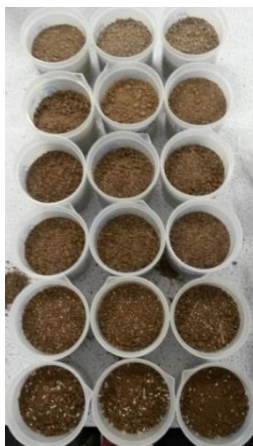


Figure 1. Chitin fraction treatments in soil mesocosms, increasing in particle size from top to bottom.

Results and Summary of Findings

Effect of 1, 5 and 10% chitin biostimulant treatments

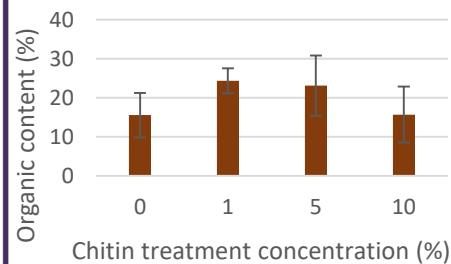


Figure 2. Soil organic matter (SOM) percentage of chitin treatments in soil mesocosms after 28 days at 1, 5 and 10% concentrations plus a control.

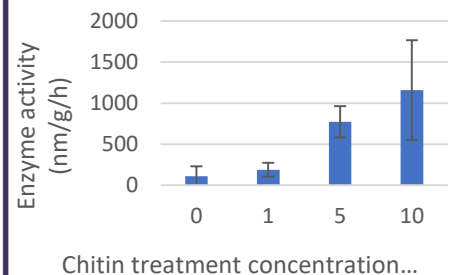


Figure 4. Chitinase enzyme activity of chitin treatments in soil mesocosms after 28 days at 1, 5 and 10% concentrations plus a control.

An increase in chitin concentration, a key soil health parameter, saw no significant difference in soil organic matter (SOM; $\text{Chi}^2 = 4.5792$, $\text{df} = 3$, $\text{p-value} = 0.2053 > 0.05$, see figure 2), although 1 and 5% were highest. There was a strong significant difference between phenol oxidase activity and chitin concentration ($F_{3, 12} = 8.027$, $\text{p-value} = < 0.01$, see figure 3), with the control and 1% most different to 5 and 10%. A significant difference in chitinase enzyme activity was seen, stimulated most under high chitin concentration ($\text{Chi}^2 = 11.735$, $\text{df} = 3$, $\text{p-value} = < 0.05$, see figure 4), with 5 and 10% having greatest activity. pH had a significant difference in reaction to chitin, with 5 and 10% slightly acidic ($\text{Chi}^2 = 8.2356$, $\text{df} = 3$, $\text{p-value} = < 0.05$, see figure 5).

Summary

Analysis indicated the ability of phenol oxidase and chitinase to mobilise recalcitrant carbon to labile compounds. Smaller particle sizes, particularly <63_m showed increased substrate utilisation, SOM and pH stability. The control and 1% concentration had most microorganism activity. GHG analysis indicates that chitin at smaller fractions and with increasing concentration may stimulate hyphal growth via an enhanced ability to translocate retained nutrients. Further exploration with SEM shows that larger fractions could maintain diversity in ped size and greater surface area for a healthy lattice soil structure and biodiverse microbial community. Future studies may include plant growth and other soil parameters for farm nutrient life-cycle analyses.

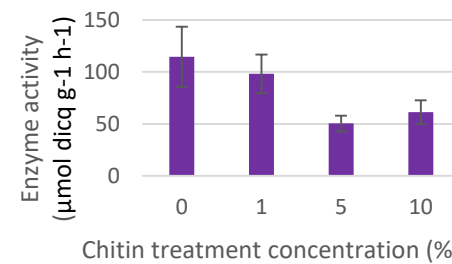


Figure 3. Phenol oxidase enzyme activity of chitin treatments in soil mesocosms after 28 days at 1, 5 and 10% concentrations plus a control.

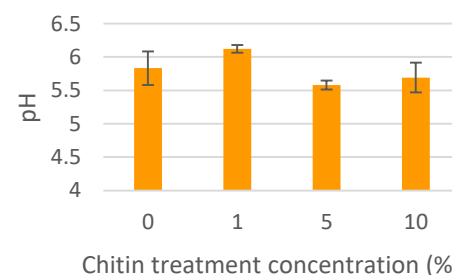


Figure 5. pH of chitin treatments in soil mesocosms after 28 days at 1, 5 and 10% concentrations, plus a control.

Effect of <63 – 2000µm chitin biostimulant particle size fractions at 5% concentration

No significant difference in SOM was seen ($\text{Chi}^2 = 10.838$, $\text{df} = 5$, $\text{p-value} = > 0.05$, see figure 6), however peaks at <63µm and 250-500µm occurred and the p-value was close to the 95% confidence threshold. Microorganism respiration of <63µm using phenol oxidase was double that of other particle size fractions, with a significant difference ($\text{Chi}^2 = 15.363$, $\text{df} = 5$, $\text{p-value} = < 0.05$, see figure 7). There was a significant difference in chitinase activity between particle sizes ($F_{5, 12} = 5.448$, $\text{p-value} = < 0.05$, see figure 8). Larger particle sizes 2000-250µm saw the greatest difference to <63µm particles. A strong difference occurred between <63µm and moderate sizes such as 500µm. There was a significant difference in pH between treatments ($\text{Chi}^2 = 13.667$, $\text{df} = 5$, $\text{p-value} = < 0.05$, see figure 9). <63µm shows a neutral pH, whereas all other treatments are acidic by a log value of 1 or more.

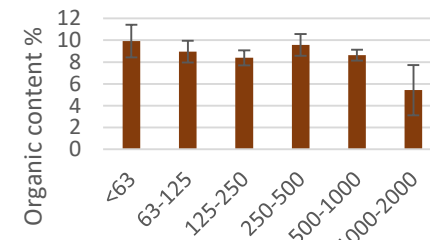


Figure 6. Soil organic matter (SOM) percentage of chitin fractions from <63 - 2000µm in soil mesocosms after 28 days.

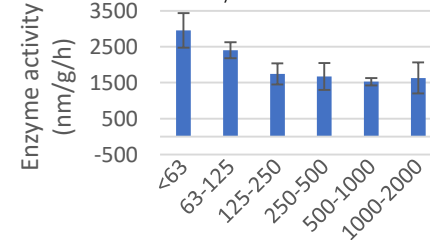


Figure 8. Chitinase enzyme activity of chitin fractions from <63 - 2000µm in soil mesocosms after 28 days.

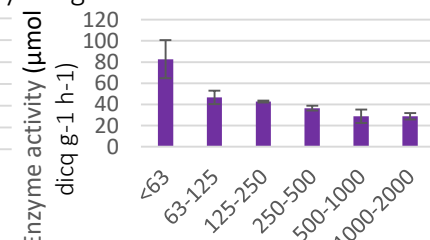


Figure 7. Phenol oxidase enzyme activity of chitin fractions from <63 - 2000µm in soil mesocosms after 28 days.

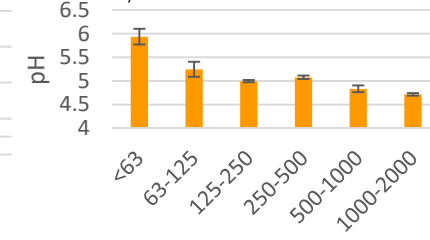


Figure 9. PH of chitin fractions in soil mesocosms from <63µm - 2000µm after 28 day incubation.

Aims and Objectives

A- Project title and brief outline of what was explored.

B- An introduction on why the project was done asnd the environmental issues faced.

Method

C- Why chitin was chosen and its useful properties and functions within the soil ecosytem in rektion to microbe communities.

D- A brief overview of the study soil and site and techniques used.

E- Wider inferences of the results with future research and applications.

Results and Summary of Findings