Oceland Biologicals

Microbial solutions for Agriculture and the Environment

A novel plant biostimulant for high value vegetable crops

Final Report

2018

Submitted to

Horticulture Nova Scotia

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EXECUTIVE SUMMARY

The effect of a novel microbial metabolite-based plant bio-stimulant product was studied on two high value vegetable crops, cauliflower and broccoli. Three laboratory experiments were conducted: 1) Petri dish germination experiment, 2) Cell pack early growth experiment and 3) biochemical analysis experiment. Additionally, three types of field experiments were completed within the 2017 field season, with emphasis on cauliflower yield enhancement. In all laboratory experiments, under varying salinity stress, conditions both broccoli and cauliflower seeds had enhanced germination or growth when treated with the bio-stimulant product as compared to untreated control. In the Petri dish experiment, the optimal concentration of the product that gave the best result was 1 %. In the cell pack experiment the concentration appears differ between formulations of the bio-stimulant product: for formulation A, the lower concentration (0.01 %) has better efficacy for dry weight whereas in formulation B, the higher concentration (0.1 %) was better. In the field, where the only test crop was cauliflower, the product improved the marketable yield through all modes of application in both concentrations (0.1 and 0.01 %) as compared to the untreated control. The greatest marketable yield was obtained in the combination application of drench and foliar spray of the lower concentration (0.01 %) of the bio-stimulant product. Further abiotic stress experiments are being conducted in the laboratory, and expanded field studies with more test crops will be conducted in the 2018 field season.

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INTRODUCTION

The use of soil amendments with agricultural crops is inevitable in today's world based on the nature of industrialized (intensive) agriculture. Many factors contribute to the necessity of these soil amendments, and these factors can all be linked. Global agricultural land accounts for approximately one-third of global land area, while arable land constitutes approximately 10.9 % of that agricultural land area (Food and Agriculture Organization, 2017a). This is a relatively small portion of land to rely on for agricultural commodities. In addition, although growth in more developed countries has slowed, the global population continues to rise, giving way to increasing demand for agricultural commodities as well as land itself. To meet the demands of a growing population, the current goal of intensive agricultural operations is to produce the greatest yield on the least amount of land while creating the greatest profit. This is most-often achieved using synthetic chemicals (i.e. pesticides and fertilizers) that have numerous negative side-effects (to humans and the environment) in addition to the target impact of increasing crop yield.

A more sustainable, environmentally conscious soil amendment to use as a replacement to synthetic chemicals is a bio-stimulant. A bio-stimulant is a direct derivation of the word: generally, it is a biological substance that has a positive effect on its host (in this work, agricultural crops) through one of many different modes of action (Calvo et al., 2014; du Jardin, 2015). The global bio-stimulants market was valued at $1.97 billion in 2016 and is estimated to grow to $3.3 billion by 2021 (Market Data Forecast Inc., 2017), showing another valuable reason for consumers, producers and researchers to convert to the more sustainable option.

The objectives of this research project were,

1. To evaluate the germination rate of cauliflower and broccoli seeds under abiotic stress in Petri dishes when treated with the novel microbial metabolite-based plant bio-stimulant product.

2. To evaluate the early vegetative growth of cauliflower and broccoli under abiotic stress in cell packs when treated with the bio-stimulant product.

3. To investigate various biochemical assays of the bio-stimulant product on broccoli and cauliflower at different timepoints.

4. To evaluate the bio-stimulant product at a larger-scale in a commercial agricultural setting.

To carry out this research, Oceland Biologicals Limited of Truro, Nova Scotia, Canada conducted four types of laboratory experiments within the year two of this project: 1) Petri dish germination experiment, 2) Cell pack early growth experiment and 3) Biochemical analysis experiment. Additionally, three types of field experiments were completed within the 2017 field season, with specific emphasis on cauliflower yield. The primary purpose of all experiments was to investigate the activity and efficacy of the Oceland Biologicals Limited proprietary novel microbial metabolite-based plant bio-stimulant (product) on two vegetable crops, broccoli and cauliflower, under salinity stress. By testing the product under abiotic stress conditions such as salinity stress, the results can be related to similar conditions present in agricultural soils due to climate change. The results are discussed in terms of identifying the effect of the proprietary product against an untreated control.

MATERIALS AND METHODS

2.1 Proprietary Novel Microbial Metabolite-Based Plant Bio-Stimulant (Product)

Two formulations of the proprietary novel microbial metabolite-based plant bio-stimulant (herein referred to as product) were used throughout the duration of the project. These formulations were manufactured (using a proprietary process) at Oceland Biologicals Limited's research facility in the Perennia Innovation Centre in Bible Hill, Nova Scotia, Canada.

2.2 Petri Dish Experiment

Cauliflower (MinuteMan) and broccoli (Green Magic) seeds (Halifax Seed Co., Halifax, NS, Canada) were placed on Watman™ filter paper in Petri dishes (ten seeds per dish, three dishes per treatment). Each dish was treated with 4 mL of treatment solution (treatments listed in Table 1), marking the beginning of the experiment. In total, seed germination was recorded from eighteen to ninety-six hours post-treatment. Firstly, germination was recorded at three-hour intervals starting eighteen hours post-treatment. Then, at thirty-six hours post-treatment, germination was recorded at six-hour intervals and finally at forty-eight hours post-treatment, germination was recorded at twelve-hour intervals. Shoot and root lengths were measured after ninety-six hours. This experiment was repeated in triplicate.

Table 1. Concentrations of product (both formulations) and salt used in treatments for the seed germination experiment.

Treatment	Concentration of Product (%)	Concentration of Salt (mM)
Water	-	-
Salt 100	-	100
Salt 200	-	200
A	1	-
B	0.1	-
C	0.01	-
A + Salt 100	1	100
B + Salt 100	0.1	100
C + Salt 100	0.01	100
A + Salt 200	1	200
B + Salt 200	0.1	200
C + Salt 200	0.01	200

2.3 Cell Pack Experiment

Cell packs with small drainage holes were filled with PRO-MIX® BX (Halifax Seed Co., Halifax, NS, Canada) and placed in seed trays without drainage. Cauliflower (MinuteMan) seeds (Vesey's Seeds, York, PE, Canada) and broccoli (Green Magic) seeds (Halifax Seed Co., Halifax, NS, Canada) were seeded approximately 1 cm into the PRO-MIX® (eight seeds per cell) and left to germinate in the dark for three to four days. Once the seeds started to germinate, the plants were thinned to one plant per cell and placed under normal growing conditions on growth racks (16/8 hours day/night). Plants were treated with treatment solution and then treated with salt solution 48 hours post-treatment. Additionally, another set of plants were treated with treatment two weeks and three weeks after germination. Leaf area and dry weight were measured one week, two weeks and three weeks after salt treatment. This experiment was repeated in triplicate.

Table 2. Concentrations of product (both formulations) and salt used in treatments for cell pack experiment.

Treatment	Concentration of Product (%)	Concentration of Salt (mM)
Water	-	-
Salt 150	-	150
A	0.001	-
B	0.0001	-
A + Salt 150	0.001	150
B + Salt 150	0.0001	150

2.4 Biochemical Analysis Experiment

Cell packs with small drainage holes were filled with PRO-MIX® BX (Halifax Seed Co., Halifax, NS, Canada) and placed in seed trays without drainage. Cauliflower (MinuteMan) and broccoli (Green Magic) seeds (Halifax Seed Co., Halifax, NS, Canada) were seeded approximately 1 cm into the PRO-MIX® (one seed per cell) and grown for one month under normal growing conditions on growth racks (16/8 hours day/night). Plants were not watered three to four days prior to treatment to maximize treatment uptake. Two experimental plans were followed for this project, the first experimental plan was repeated in duplicate, the second experimental plan (with the addition of salt) was completed once within the timeframe of this report.

Table 3. Concentrations of product used in treatments for biochemical analysis experiments.

Treatment	Concentration of Product (%)	Concentration of Salt (mM)
	Experimental Plan 1	
Water	-	-
A	1	-
B	0.1	-
C	0.01	-
	Experimental Plan 2	
A + Salt 100	1	100
B + Salt 100	0.1	100
C + Salt 100	0.01	100
A + Salt 200	1	200
B + Salt 200	0.1	200
C + Salt 200	0.01	200

For the first experimental plan, three leaves from three individual plants were collected as one sample, and a sample plant was never sampled more than once. Leaf samples were collected at 24, 48 and 72 hours post-treatment. For the second experimental plan, three leaves from one plant was collected as one sample. Leaf samples were collected at 24, 72 and 168 (seven days) post-treatment. Leaf samples were cut with scissors, weighed and wrapped in aluminum foil (to avoid contamination) and were flash frozen with liquid nitrogen (N_2). Frozen leaves were stored at 80 °C for further analysis.

All biochemical analyses were based on spectrophotometric detection methods and they were carried out in 96 well plates. All samples were run in a BioTek Synergy HT MicroPlate reader using Gen5$^{1.05}$ software to measure the absorbance in respective wavelengths.

2.4.1 Lipid Peroxidation

Lipid peroxidation was estimated by determining the malondialdehyde (MDA) concentration produced by the Thiobarbituric acid (TBA) reaction according to Hodges *et al.* (1999). Leaf material (≈ 0.5 g) was homogenized in 10 mL of 80 % alcohol. Samples were centrifuged at 3,000 rpm for 10 minutes at 4 °C. In the first experimental plan, two methodologies were followed for each set of samples: with the first set of samples, 1 mL of

extract was mixed with 1 mL of 0.5 % (w/v) TBA in 20 % (w/v) TCA and 0.01 % Butylated Hydroxytoluene (BHT). With the second set of samples, the same methodology was followed without the TBA. The mixture was incubated at 90 °C for 30 minutes and then cooled at room temperature. Samples were centrifuged at 4,000 rpm for 3 minutes and 200 µL of supernatant was transferred to a 96-well plate. The absorbance of the supernatant was read at 400, 532 and 600 nm.

2.4.2 Quantification of Proline

A modified microplate method was used for quantification of proline (Carillo et al., 2011). Leaf samples (≈ 0.5 g) were homogenized in liquid nitrogen and collected in 1.5 mL microfuge tubes followed by the addition of 1 mL of 70 % ethanol, then vortexed. Tubes were then centrifuged at 12,000 rpm for 15 minutes at 4 °C. Five hundred millilitres of supernatant were added with 1 mL of reaction mixture containing 1 % ninhydrin (w/v) dissolved in 60 % acetic acid (v/v) and 20 % ethanol (v/v). The tubes containing the reaction mixture were mixed, sealed and heated at 95 °C in a water bath for 30 minutes. Samples were centrifuged at 2,500 rpm for 1 minute and 200 µL of supernatant was transferred to a 96-well plate. The absorbance of the supernatant was read at 520 nm against a blank containing ethanol and the reaction mixture. The proline amount was determined using an L-proline standard curve.

2.4.2.1 Preparation of the L-Proline Standard Curve

A concentration gradient of L-Proline (concentrations ranging from 0 to 1 mM) was prepared by dissolving L-proline in 70 % ethanol. Each standard (500 µL) was reacted with 1 mL of reaction mixture. The tubes containing the reaction mixture were mixed, sealed and heated at 95 °C in a water bath for 30 minutes, and absorbance was recorded. Absorbance values were plotted against L-proline concentration to obtain the standard curve (Figure 1).

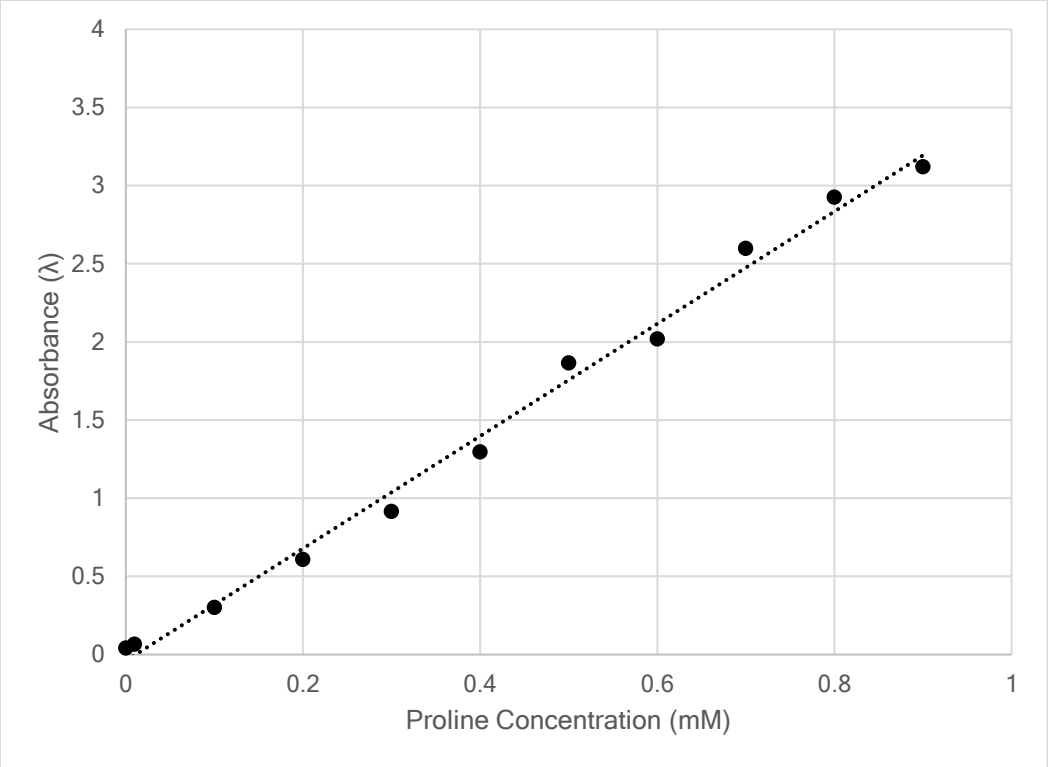

Figure 1. Proline standard curve based on spectrophotometric readings at 520 nm. The line of best fit of the graph is $y = 3.5931x + 0.0398$, $R^2 = 0.993$.

2.4.3 Soluble Sugar Estimation

Total sugars were estimated following the phenol-sulphuric acid method described by Dev (1990). Leaf samples (≈ 0.3 g) were homogenized in liquid nitrogen and collected in 50 mL falcon tubes followed by the addition of 10 mL of 90 % ethanol. Tubes were sealed and incubated in a hot water bath (60 °C) for 60 minutes. The extract was collected from the water bath and the final volume was made up to 25 mL by adding 90 % ethanol. The sample was then vortexed and centrifuged at 4,000 rpm for 3 minutes.

For estimation, a 1 mL aliquot was transferred to a thick-walled glass test tube and 1 mL of 5 % phenol was carefully added to the sample and mixed thoroughly. Five millilitres of concentrated sulphuric acid (analytical grade) was added carefully and mixed thoroughly by vertical agitation with a glass rod. The mixture was cooled at room temperature, and the absorbance was read at 485 nm against a blank containing distilled water, phenol and sulphuric acid. The amount of soluble sugar was estimated using a standard sugar curve (Figure 2).

2.4.3.1 Preparation of the Sugar Standard Curve

A concentration gradient of sucrose (concentrations ranging from 0 to 300 µg) was prepared by dissolving sucrose in distilled water using thick-walled glass test tubes. Each standard (1 mL) was reacted with the 1 mL of 5 % phenol and 5 mL of concentrated sulphuric acid as described above and absorbance at 485 nm was recorded. Absorbance values were plotted against sugar concentrations to obtain the standard curve (Figure 2).

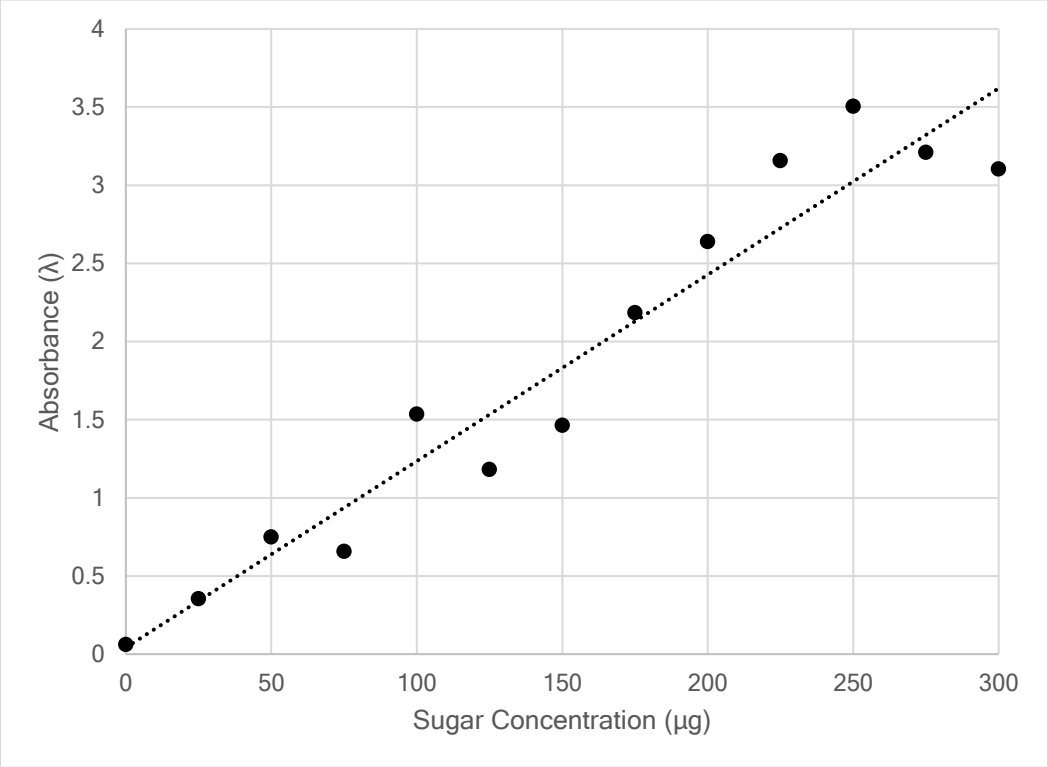

Figure 2. Sugar standard curve based on spectrophotometric readings at 485 nm. The line of best fit of the graph is $y = 0.0119x + 0.0421$, $R^2 = 0.9316$.

2.5 Field Research

2.5.1 Field Site Description

There were four unique field plots used throughout the duration of the 2017 field research season, three of which were in different fields. These fields were located within 5 km of Melvin Farms Limited, the collaborating farm located in the community of Canning, Nova Scotia, Canning (latitude 45°18'42" N, -64°40'26" W) (Google Maps, 2017a). The average summer precipitation is 428.8 mm and the average summer daily temperature is 16 °C (summer representing May – September; averages calculated from 1981 – 2010, Environment Canada, 2017).

2.5.2 Effect of OBL product on overall growth and yield of cauliflower.

2.5.2.1 Experimental Design

Cauliflower seedlings were seeded, maintained in the greenhouse and planted in the field by the commercial operator. In this experiment, the concentration of the OBL product (Table 6) was investigated in addition to the method of application of the product. Three methods of application were utilized: A) soil drench treatment, B) foliar spray treatment and C) combination soil drench/ foliar spray treatment. Treatments were arranged in a randomized completed block design with three plot replicates per treatment and application method.

Treatments were applied once every two weeks for six weeks (three treatments in total). Foliar treatments were applied using a backpack sprayer (Roundup® 4-Gal Professional Backpack Sprayer). Soil drench treatments were applied using mason jars. This experiment was repeated twice, with both formulations being used in both experiments.

Table 4. Concentrations of product (both formulations) used in treatments for Experiment #3.

Treatment Abbreviation	Product Concentration (%)
Water	-
B	0.1
C	0.01

*Tween®20 was added to the backpack sprayer to ensure stabilization of the treatment on plant leaves.

2.5.2.2 Growth Measurements

Once treatments are finished, the cauliflower plants are left to grow until the flower (the cauliflower head) has matured to commercial size). At this time, marketable yield is determined by counting the number of cauliflower heads per treatment. Marketability is based on numerous factors, such as colouration, and size. In addition to marketable yield (a non-destructive measure), ten cauliflower heads from each plot were harvested in the second experiment to determine mean cauliflower head weight per plot and per treatment.

2.6 Statistical Methods

All analyses were performed using RStudio Version 1.1.383, JAGS Version 4.3.0 and R Version 3.4.2.

RESULTS

3.1 Petri Dish Experiment

Three concentrations of the product (both formulations) alone, as well as in combination with two combinations of salt (100 and 200 mM salt) (Table 1) were applied to broccoli and cauliflower seeds.

3.1.1 Seed Germination

Seed germination was measured as described in the methodology: at three-hour intervals starting eighteen hours post-treatment, then at six-hour intervals thirty-six hours post-treatment and finally at twelve-hour intervals forty-eight hours post-treatment. A seed was counted as germinated when the radicle length was half the length of the seed.

3.1.1.1 Broccoli

There was a vast effect of salinity on the germination rate of broccoli seeds, as shown in Figures 3 through 8. The presence of salt (regardless of concentration) shows a definitive lag time in the germination rate of broccoli seeds in comparison to those in the absence of salt (Figures 4 and 6 versus Figure 3).

Between treatments, it appears that the higher concentration of the product provided the greatest effect against salinity stress of the concentrations tested. Seed germination for seeds treated with concentration A (1 %) + Salt 100 mM (S100) was significantly greater than concentration C (0.01 %) + S100 at 21 hours post-treatment ($P < 0.05$) (Figure 5). Under higher salinity conditions, A (1 %) + S200 was significantly greater than the S200 control and the C (0.01 %) + S200 concentration 27 hours post-treatment and both B (0.1 %) + S200 and C (0.01 %) + S200 concentrations 30 hours post-treatment ($P < 0.05$) (Figure 7).

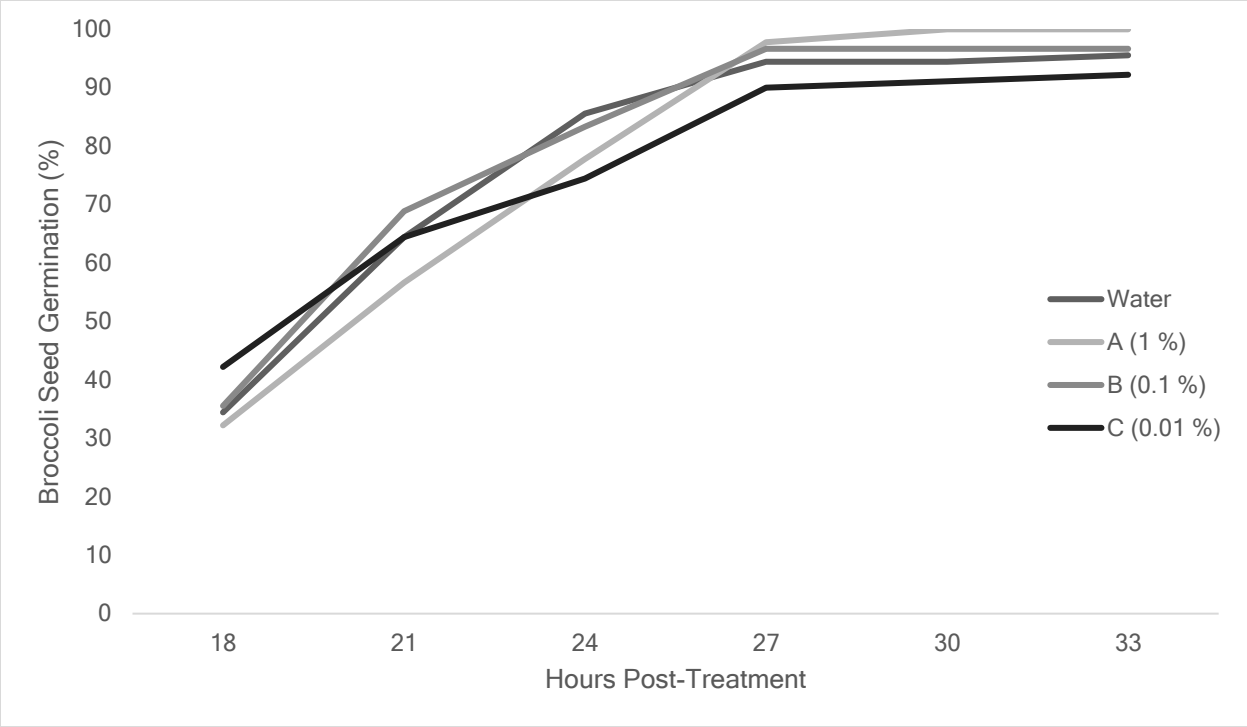

Figure 3. Mean broccoli seed germination pooled from three independent experiments using Formulation A of the product.

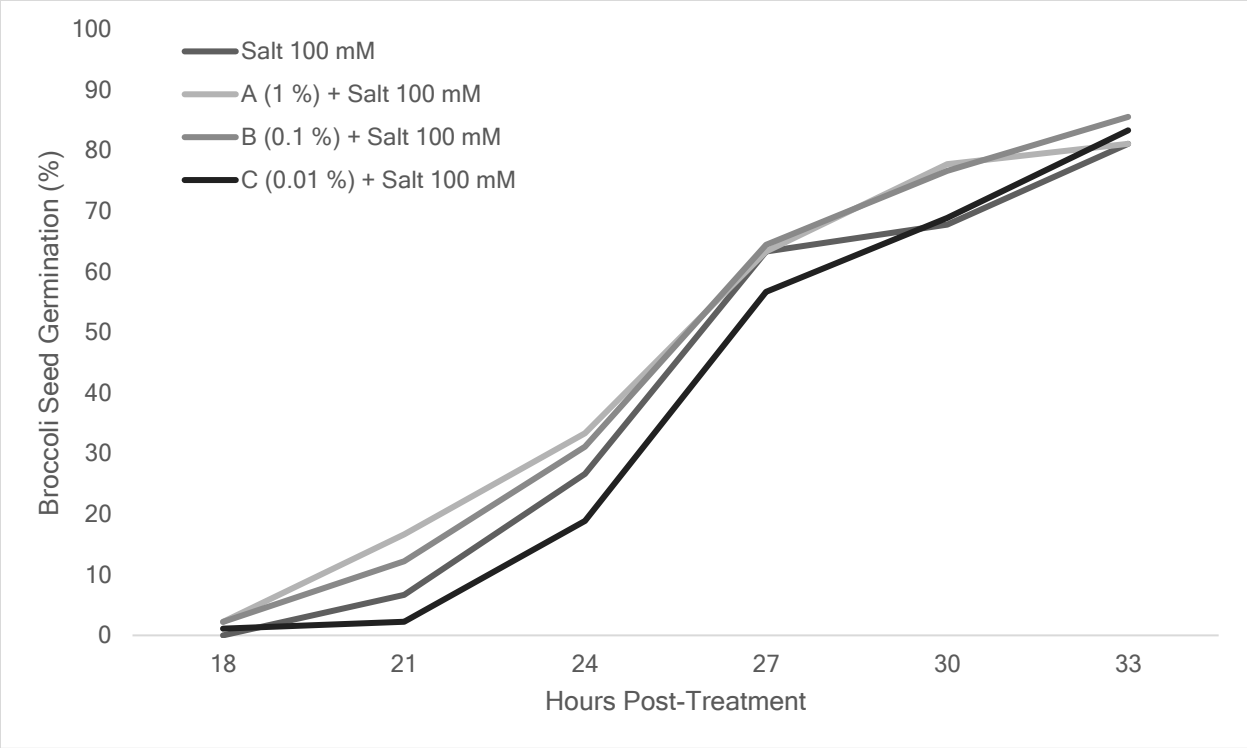

Figure 4. Mean broccoli seed germination pooled from three independent experiments using Formulation A of the product amended with 100 mM salt.

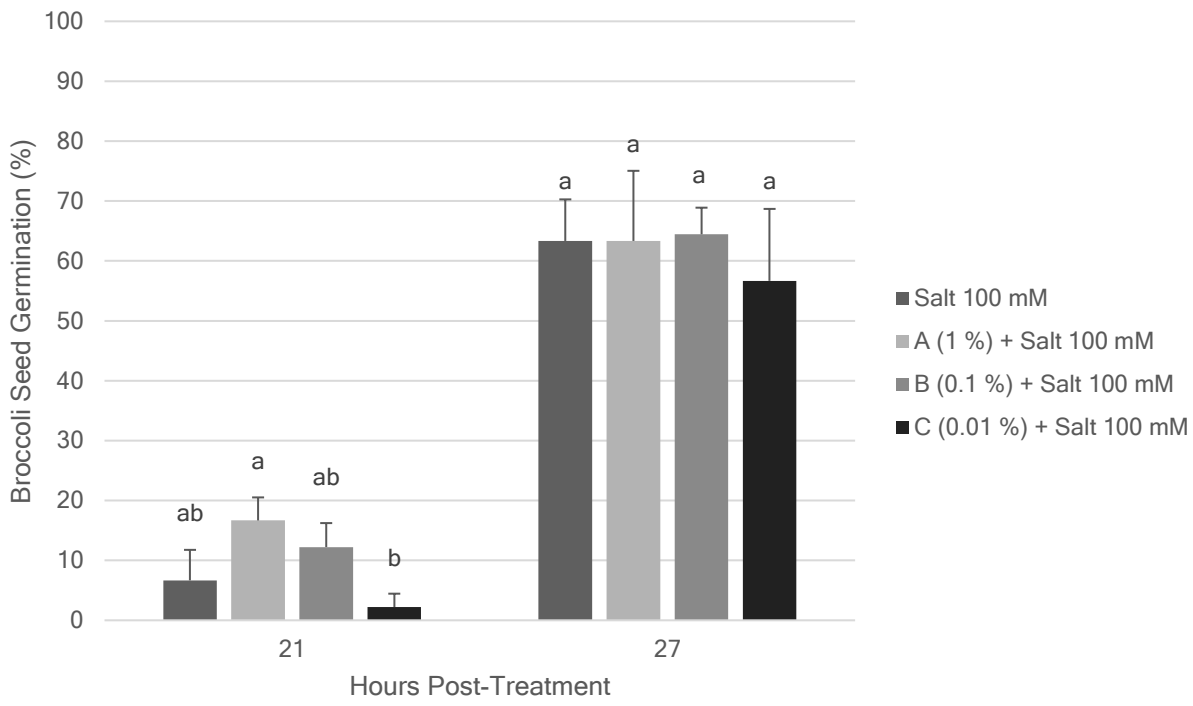

Figure 5. Mean broccoli seed germination for two time points, 21 and 27 hours post-treatment. Seed germination values were pooled from three independent experiments using Formulation A of the product amended with 100 mM salt. Different letters within each time point indicate significant difference at $P < 0.05$. Bars indicate standard error.

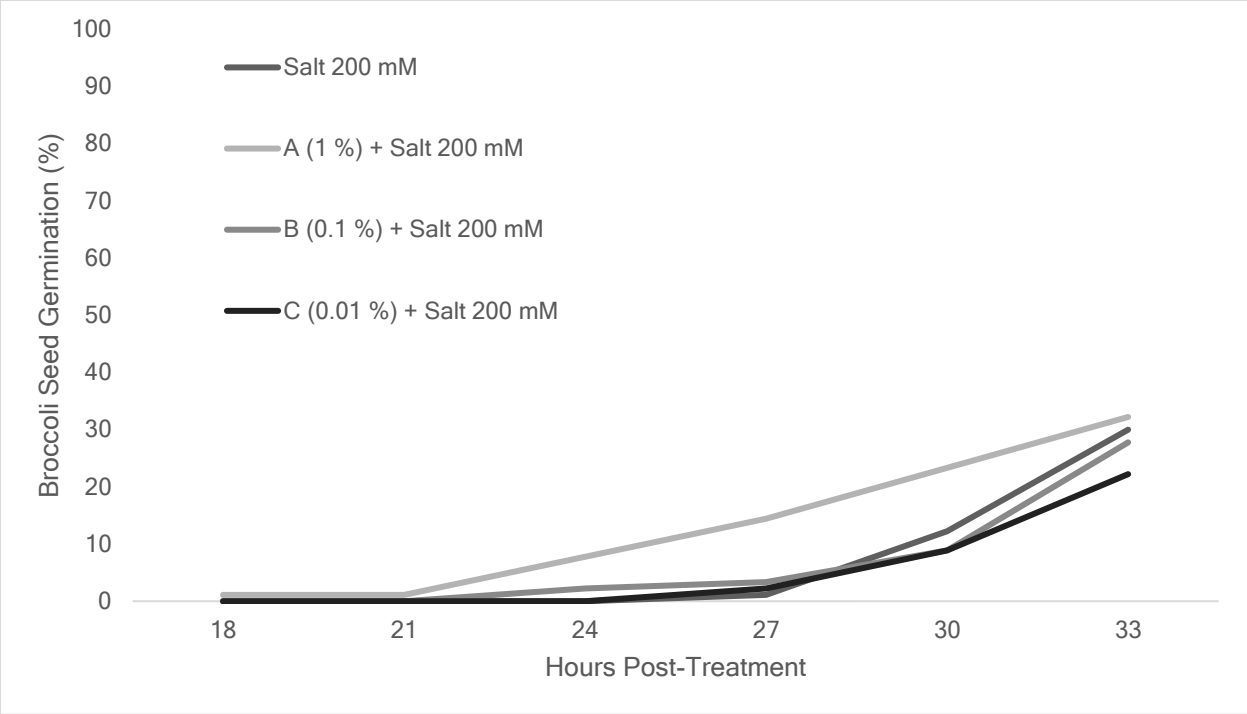

Figure 6. Mean broccoli seed germination pooled from three independent experiments using Formulation A of the product amended with 200 mM salt.

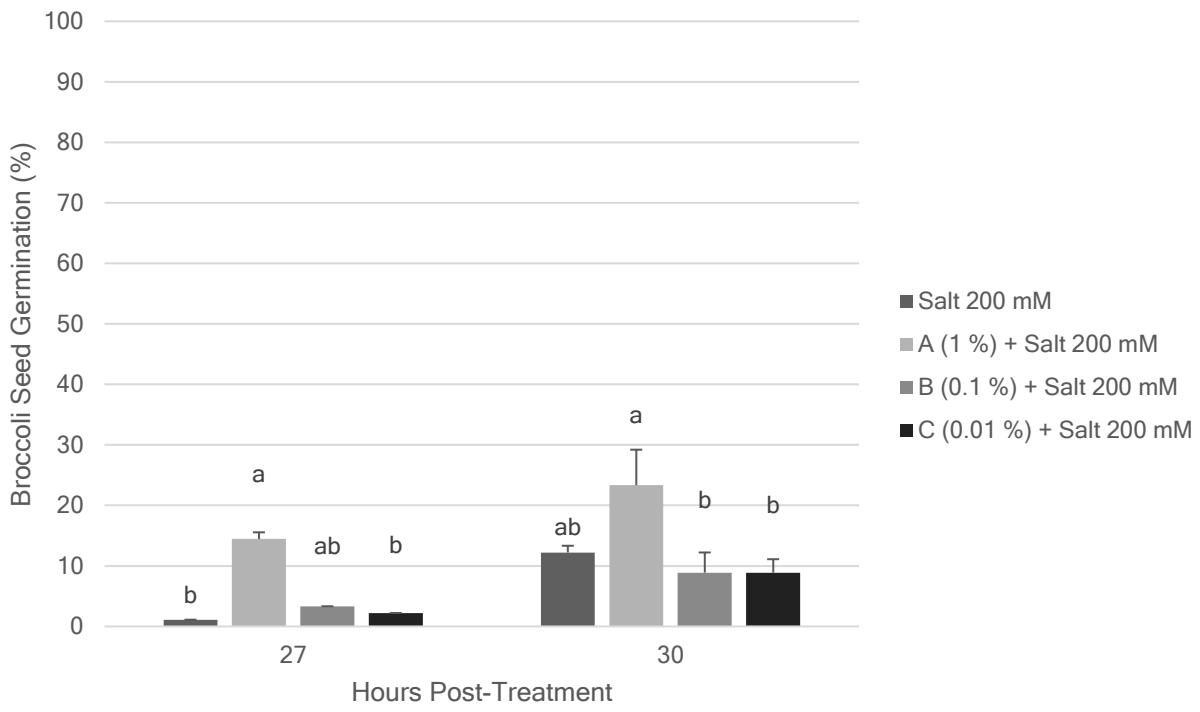

Figure 7. Mean broccoli seed germination for two time points, 27 and 30 hours post-treatment. Seed germination values were pooled from three independent experiments using Formulation A of the product amended with 200 mM salt. Different letters within each time point indicate significant difference at $P < 0.05$. Bars indicate standard error.

3.1.1.2 Cauliflower

There was an even greater effect of salinity on the germination rate of cauliflower seeds compared to broccoli, as shown in Figures 8 through 12. The presence of salt (regardless of concentration) showed a definitive lag time in the germination rate similar to broccoli seeds in comparison to those in the absence of salt (Figures 9 and 11 versus Figure 8), however the 200 mM salt greatly reduced the initial germination of the cauliflower seeds. The broccoli seeds showed some (although minimal) germination in the early hours post-treatment under the highly saline conditions, however, there was no germination in the cauliflower seeds under the same saline conditions until 36 hours post-treatment (Figure 11).

There were no statistically significant differences to identify a difference between any of the treatments under any of the three experimental conditions. By looking at the trend in the graphs, it appears that a similar trend is occurring in the cauliflower seed germination as was in the broccoli seed germination: seeds treated with concentration A (1 %) under saline conditions show greater germination than the lower treatment concentrations (Figures 10 and 12).

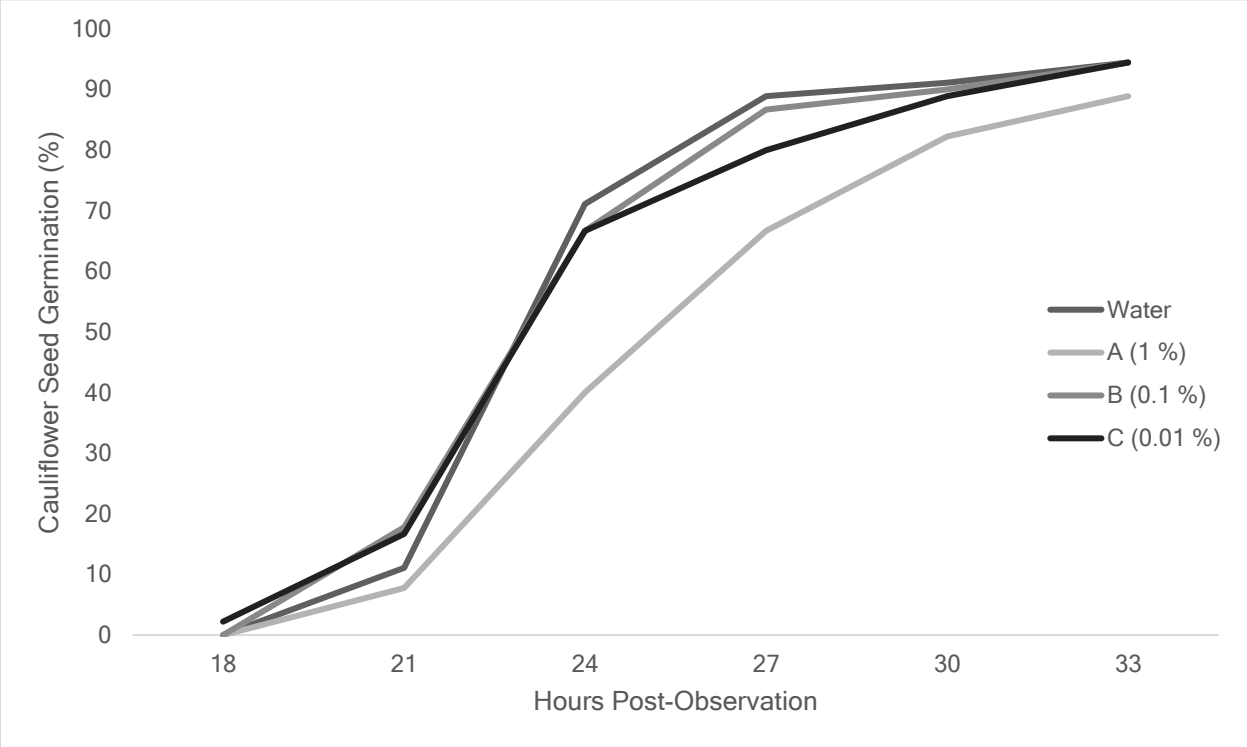

Figure 8. Mean cauliflower seed germination pooled from three independent experiments using Formulation A of the product.

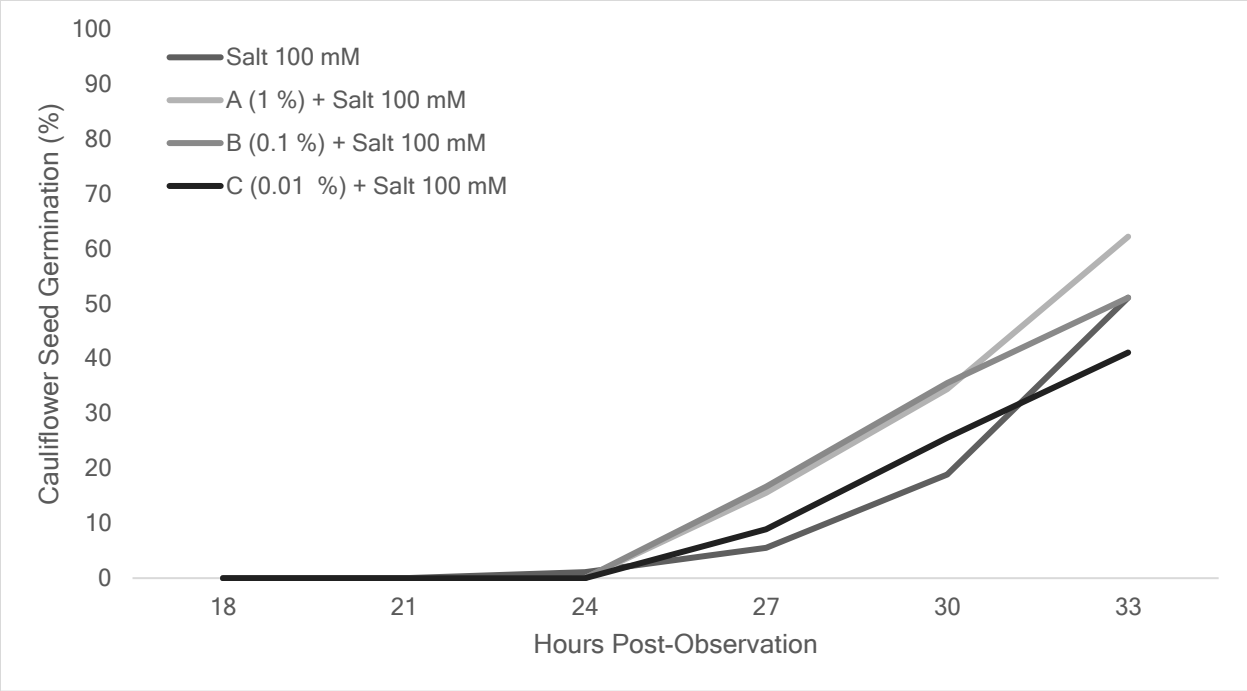

Figure 9. Mean cauliflower seed germination pooled from three independent experiments using Formulation A of the product amended with 100 mM salt.

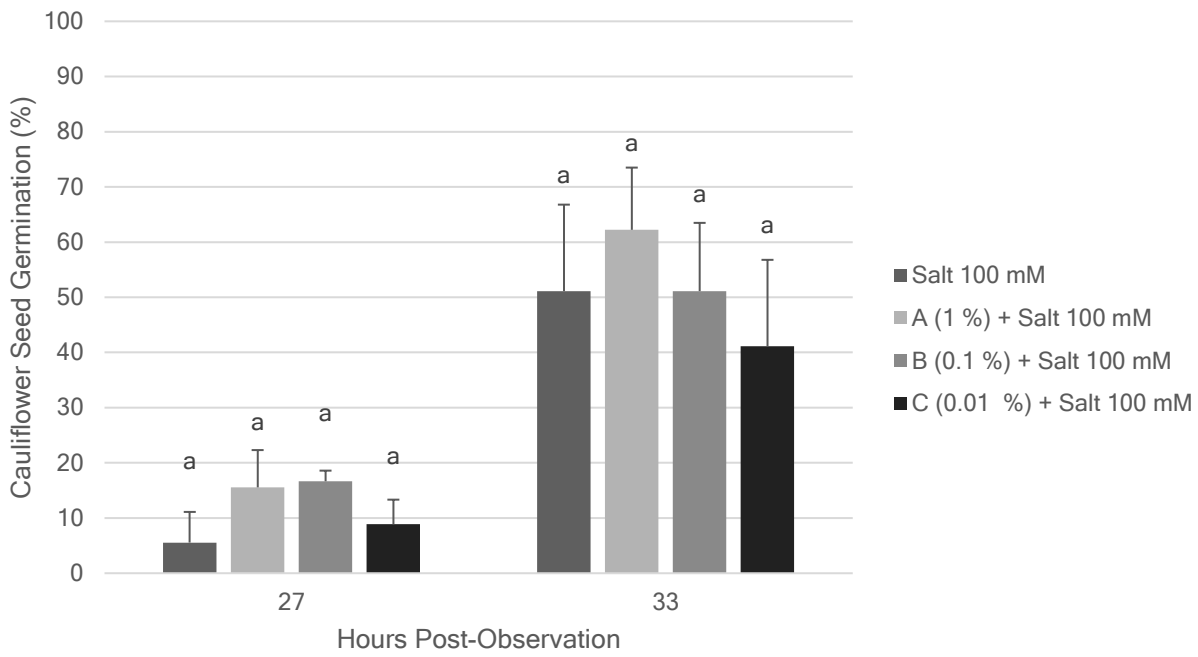

Figure 10. Mean cauliflower seed germination for two time points, 27 and 30 hours post-treatment. Seed germination values were pooled from three independent experiments using Formulation A of the product amended with 100 mM salt. Different letters within each time point indicate significant difference at $P < 0.05$. Bars indicate standard error.

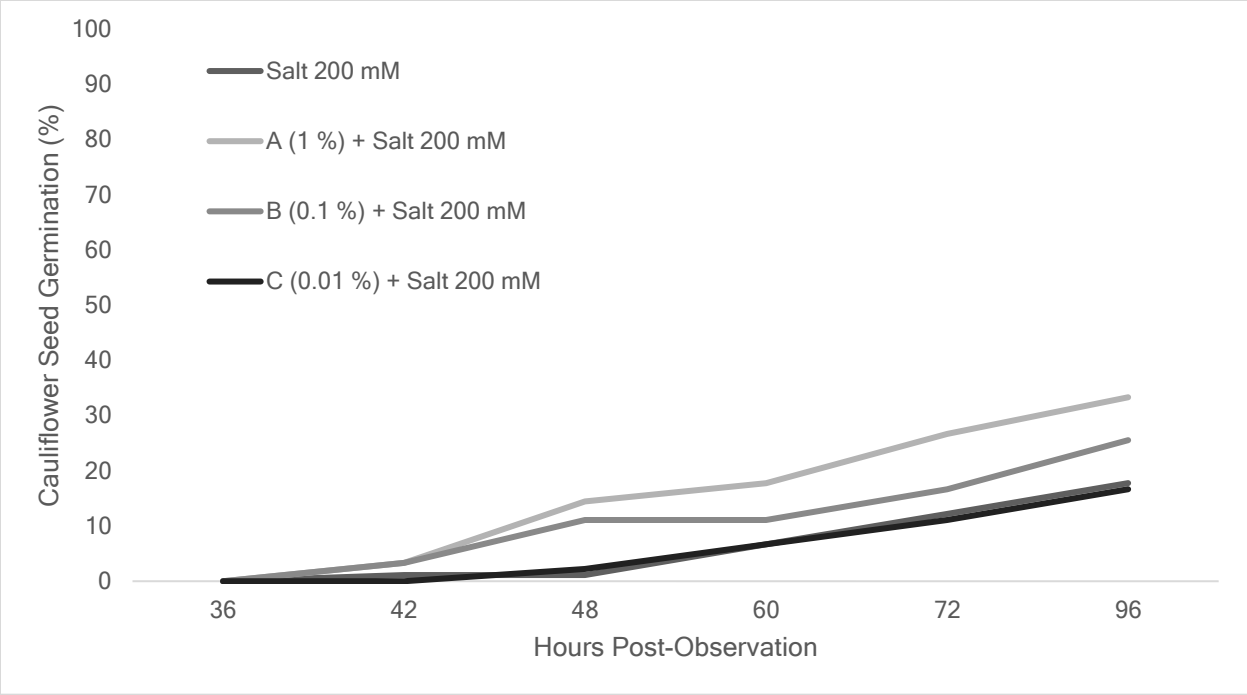

Figure 11. Mean cauliflower seed germination pooled from three independent experiments using Formulation A of the product amended with 200 mM salt.

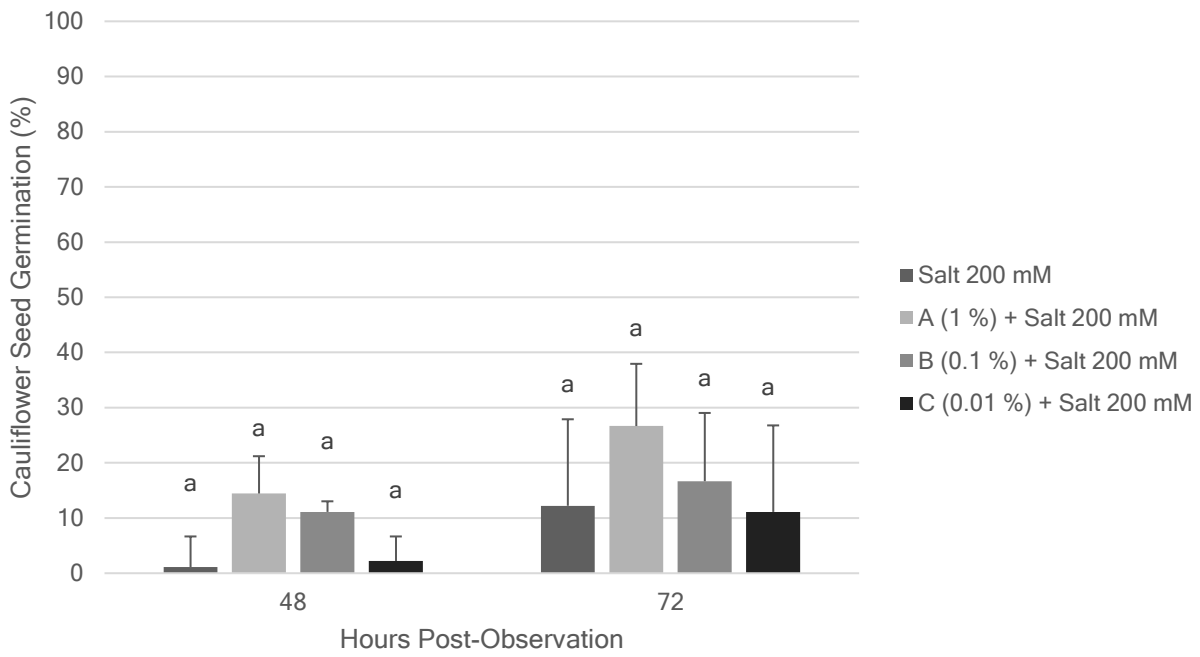

Figure 12. Mean cauliflower seed germination for two time points, 48 and 72 hours post-treatment. Seed germination values were pooled from three independent experiments using Formulation A of the product amended with 200 mM salt. Different letters within each time point indicate significant difference at $P < 0.05$. Bars indicate standard error.

3.2 Cell Pack Experiment

3.2.1 Leaf Area

Leaf area was measured using an EPSON scanner and WinFOLIA™ software. Leaves were scanned one week, two weeks and three weeks post-germination (also after one, two and three respective treatments).

3.2.1.1 Broccoli

There was little effect of either formulation of the product on broccoli leaf area as compared to the control with respect to time (Figure 13). The greatest differences in leaf area between treated plants and control plants occurred after the first harvest, and these were the only statistically significant results throughout the entire experiment. Between formulations, there is no difference between formulation A and B in comparison to each other. Between treatment concentrations, in both formulations, it appears that after harvest 1, the higher concentration (1/1,000, or 0.1 %) had a greater leaf area than its lower counterpart (1/10,000, or 0.01 %). This trend changed in harvests 2 and 3.

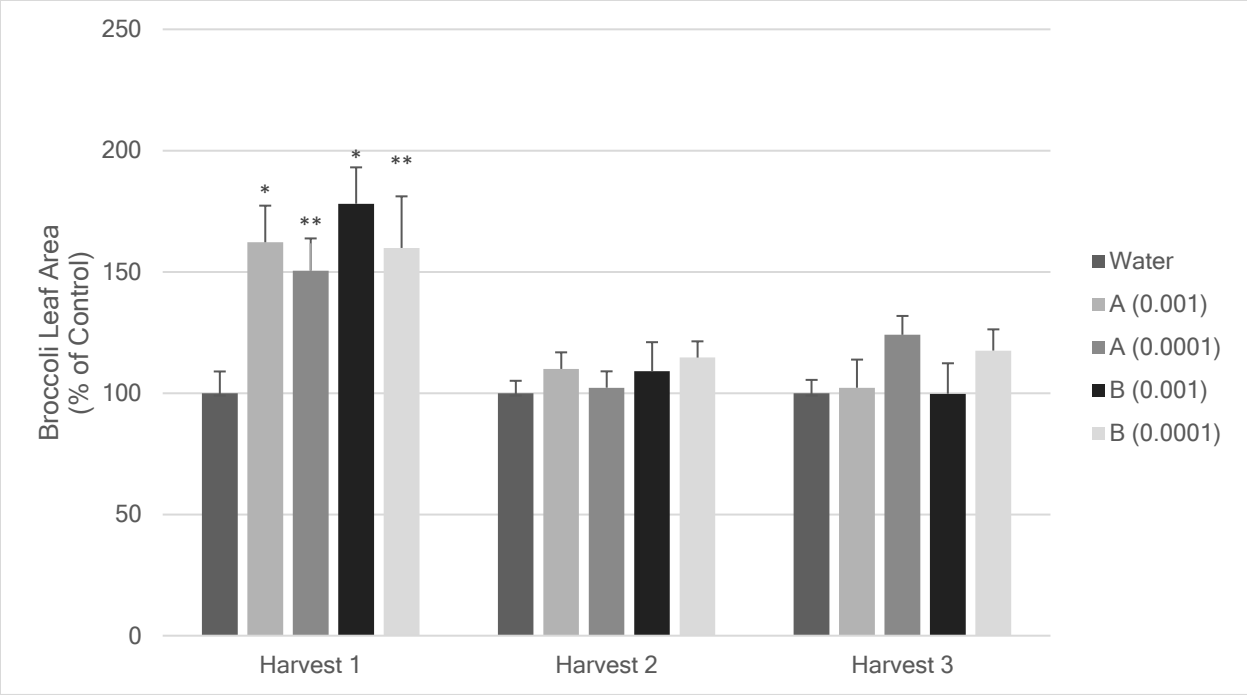

Figure 13. Mean broccoli leaf area (% of control) for three time points, one, two and three weeks post-treatment. Leaf area values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the control at $P < 0.01$ and two asterisks (**) indicates significant difference from the control at $P < 0.05$ within one time point. Bars indicate standard error.

There was a greater effect on leaf area of both formulations of the product amended with 150 mM salt as compared to the control with respect to time (Figure 14). Although there were very few statistically significant results throughout the experiment, there are still interesting trends identified. Between formulations, similar to the leaf area with no salt, there is no difference between formulation A and B in comparison to each other. Between treatment concentrations, in formulation B, it appears that after harvest 1, the higher concentration (1/1,000, or 0.1 %) had a greater leaf area than its lower counterpart (1/10,000, or 0.01 %). In formulation A, it appears that the lower concentration consistently resulted in a greater leaf area than the higher concentration.

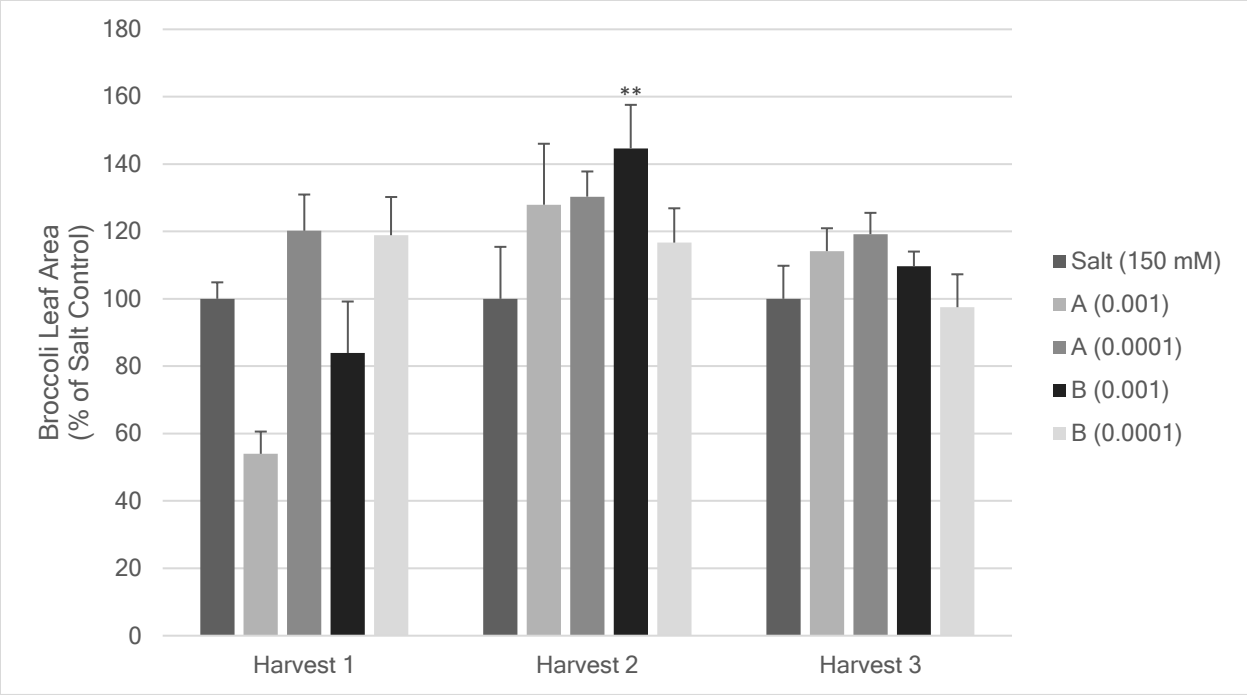

Figure 14. Mean broccoli leaf area (% of salt control) for three time points, one, two and three weeks post-treatment. Leaf area values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the salt control at $P < 0.01$ and two asterisks (**) indicates significant difference from the salt control at $P < 0.05$ within one time point. Bars indicate standard error.

3.2.1.2 Cauliflower

The effect of either formulation of the product on cauliflower leaf area as compared to the control with respect to time was very similar to that of broccoli (Figure 15). Again, similar to broccoli, the greatest differences in leaf area between treated plants and control plants occurred after the first harvest. Between formulations, formulation A appears to have greater leaf area than formulation B after harvests 1 and 2. In formulation A, it appears that after harvests 2 and 3, the higher concentration (1/1,000, or 0.1 %) had a lower leaf area than its lower counterpart (1/10,000, or 0.01 %). This trend is different in harvest 1. In formulation B, it appears that consistently, throughout all harvests, the higher concentration had a lower leaf area than its lower counterpart.

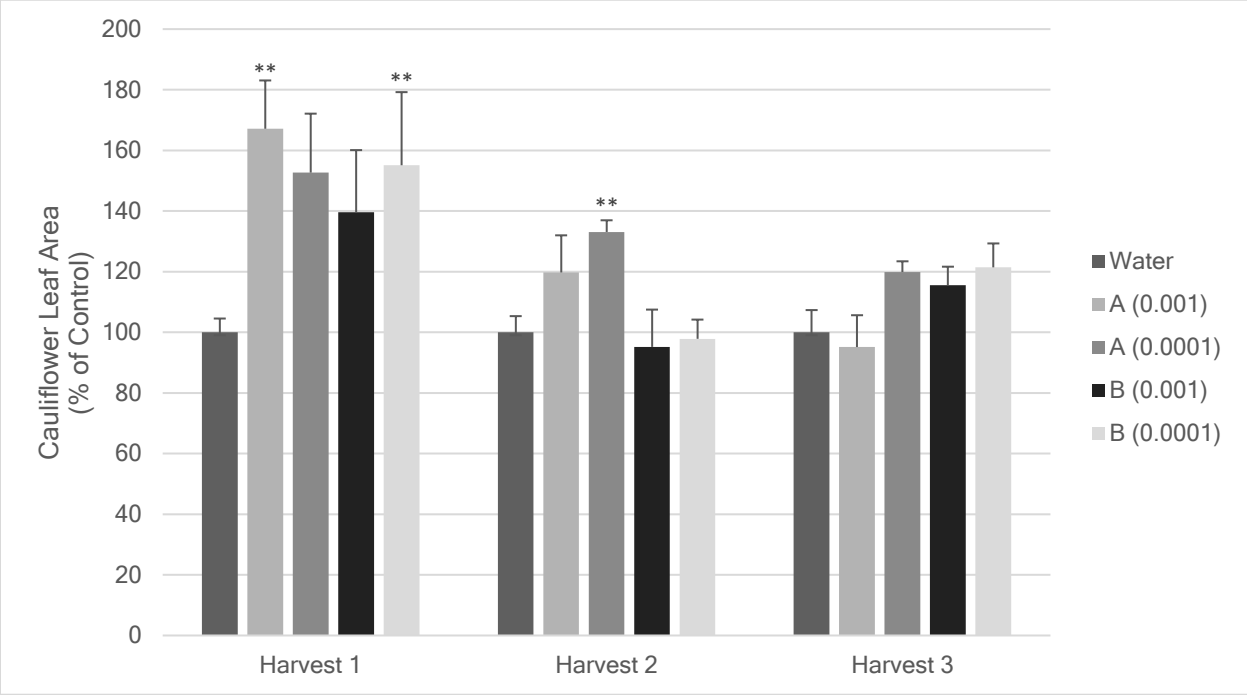

Figure 15. Mean cauliflower leaf area (% of control) for three time points, one, two and three weeks post-treatment. Leaf area values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the control at $P < 0.01$ and two asterisks (**) indicates significant difference from the control at $P < 0.05$ within one time point. Bars indicate standard error.

There was a greater effect on leaf area of both formulations of the product amended with 150 mM salt as compared to the control with respect to time (Figure 16). The products had the greatest effect on leaf area after the first harvest, and had the least effect after the third harvest, showing a potential effect with relation to plant stress. In harvests 1 and 2, all treatments but one (Formulation A, Concentration 1/1,000 or 0.01 %) resulted in statistically greater leaf areas than the salt control ($P < 0.05$).

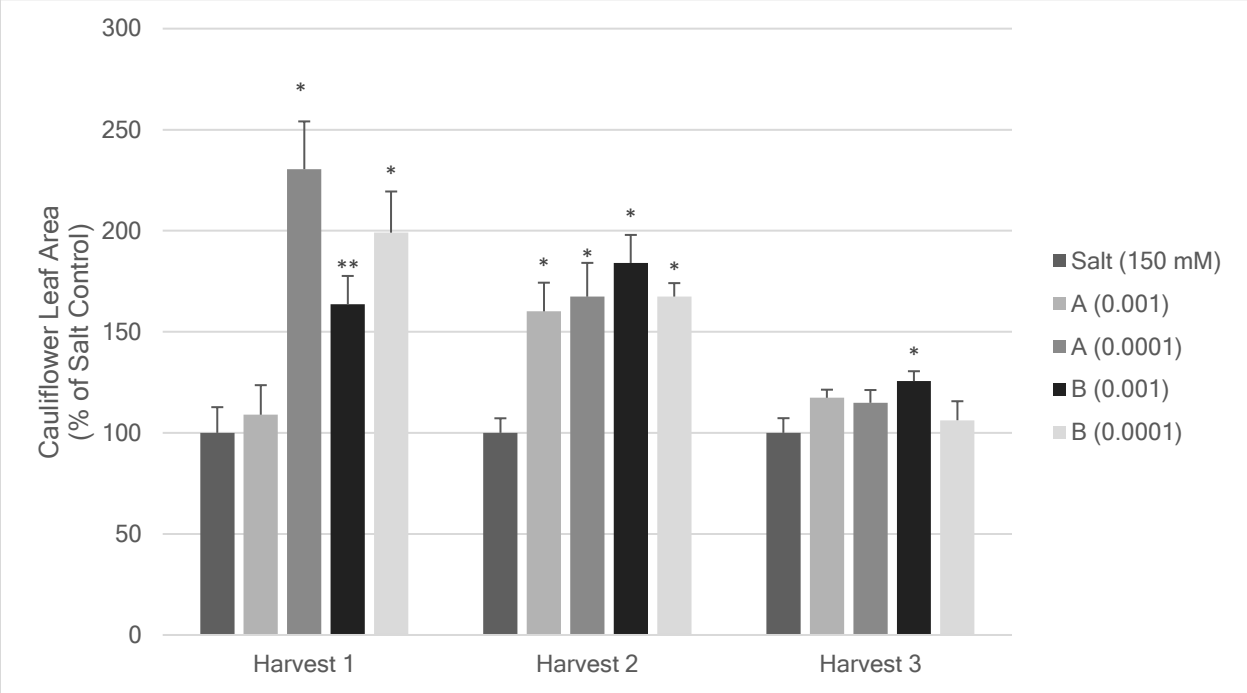

Figure 16. Mean cauliflower leaf area (% of salt control) for three time points, one, two and three weeks post-treatment. Leaf area values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the salt control at $P < 0.01$ and two asterisks (**) indicates significant difference from the salt control at $P < 0.05$ within one time point. Bars indicate standard error.

3.2.2 Dry Weight

Dry weight was measured after the harvested leaves were dried in an oven for at least 72 hours at 60 °C.

3.2.2.1 Broccoli

The broccoli dry weight was compared to the untreated control was greater after all harvests. The dry weight of plants treated with formulation A, concentration 1/10,000 (0.01 %) of the product was statistically greater than the control after all three harvests ($P < 0.05$, $P < 0.01$) (Figure 17).

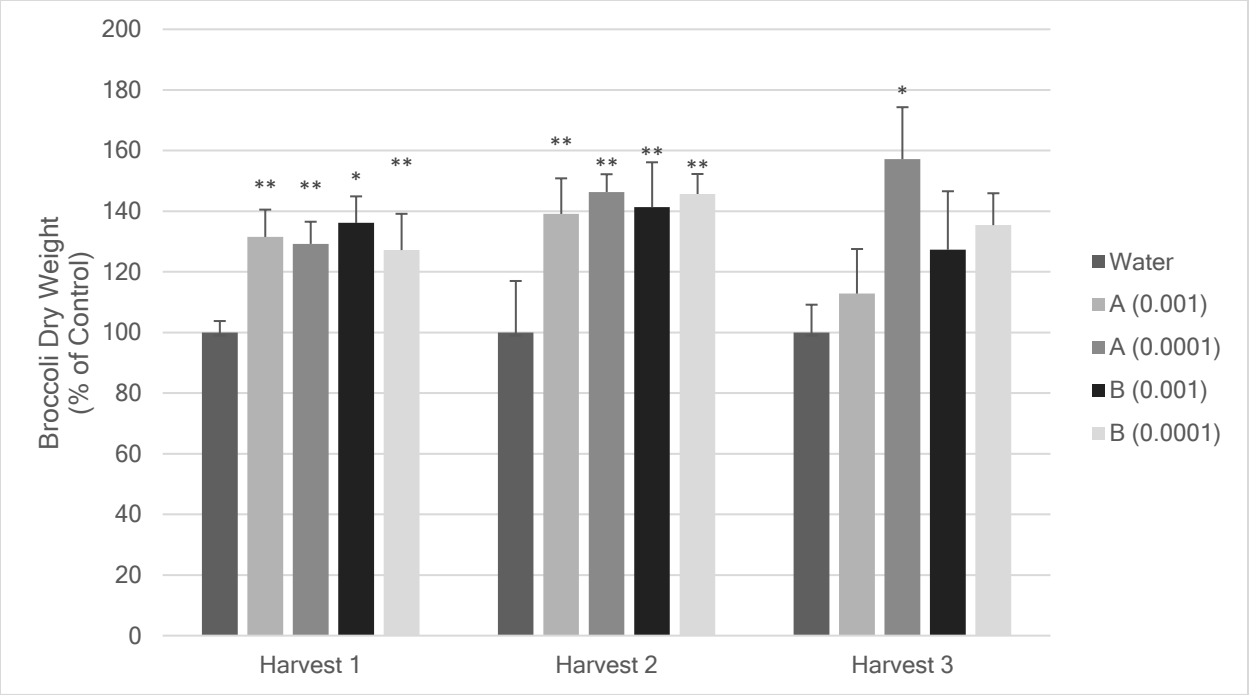

Figure 17. Mean broccoli leaf dry weight (% of control) for three time points, one, two and three weeks post-treatment. Dry weight values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the control at P < 0.01 and two asterisks (**) indicates significant difference from the control at P < 0.05 within one time point. Bars indicate standard error.

The second and third harvests showed greater dry weights compared to the first harvest (Figure 18). This corresponds to a responsive action of the product to the salt stress rather than a proactive action (i.e. the product treatment occurred before the salt treatment in the first harvest; the product treatment occurred after the salt treatment in the second and third harvests). Broccoli plants treated with formulation B, concentration 1/1,000 (0.1 %) had statistically greater dry weights than the control plants in both the second and third harvests.

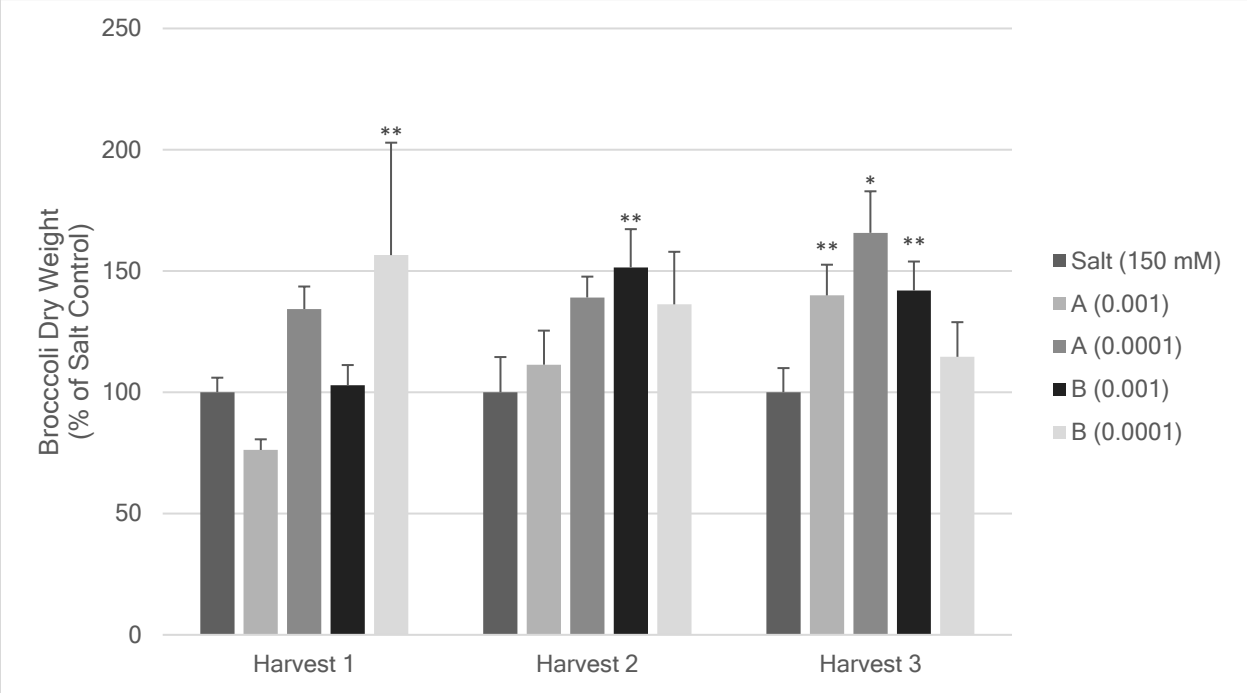

Figure 18. Mean broccoli leaf dry weight (% of salt control) for three time points, one, two and three weeks post-treatment. Dry weight values were taken from one independent experiment using both Formulations of the product amended with 150 mM salt. One asterisk (*) indicates significant difference from the salt control at $P < 0.01$ and two asterisks (**) indicates significant difference from the salt control at $P < 0.05$ within one time point. Bars indicate standard error.

3.2.2.2 Cauliflower

Cauliflower dry weight compared to the untreated control was greater after all harvests, similar to the results of broccoli. Again, consistent with the results from broccoli, the dry weight of plants treated with formulation A, concentration 1/10,000 (0.01 %) of the product was statistically greater than the control after all three harvests ($P < 0.05$, $P < 0.01$) (Figure 19).

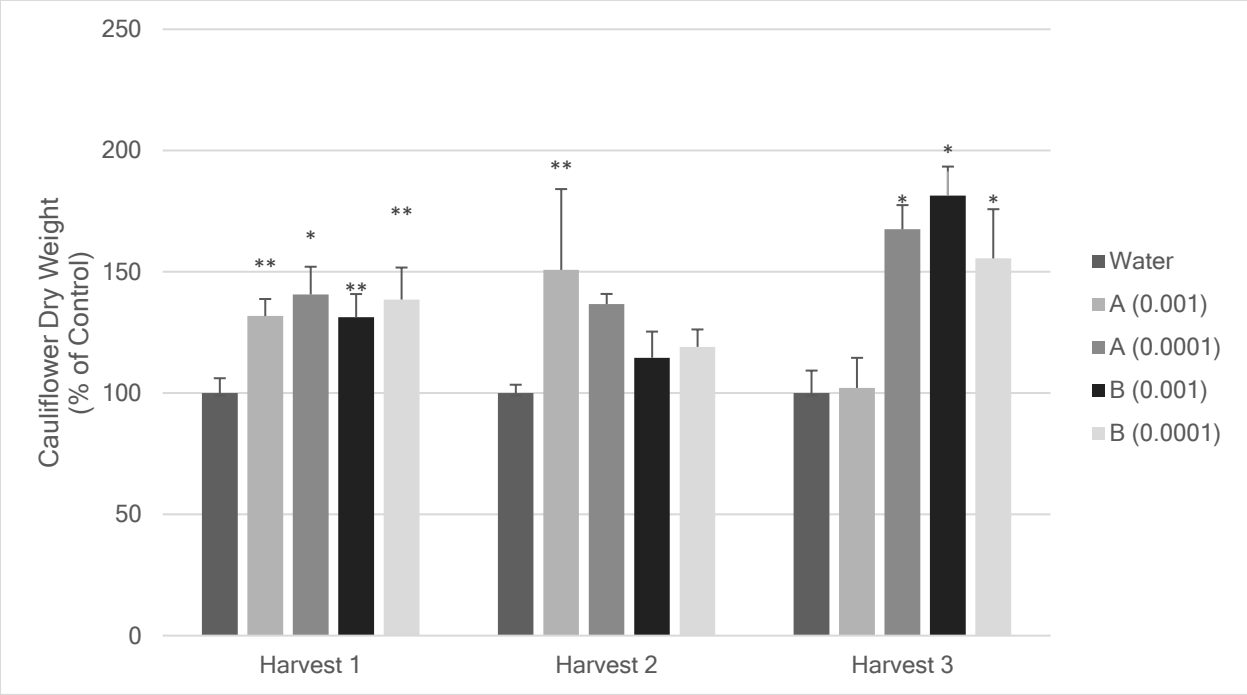

Figure 19. Mean cauliflower leaf dry weight (% of control) for three time points, one, two and three weeks post-treatment. Dry weight values were taken from one independent experiment using both Formulations of the product. One asterisk (*) indicates significant difference from the control at $P < 0.01$ and two asterisks (**) indicates significant difference from the control at $P < 0.05$ within one time point. Bars indicate standard error.

The first and third cauliflower harvests showed greater dry weights compared to the second harvest (Figure 20). Cauliflower plants treated with formulation B, concentration 1/1,000 (0.1 %) had greater dry weights than plants treated with formulation B, concentration 1/10,000 (0.01 %) in all harvests while the opposite trend occurred in formulation A (concentration 1/1,000 had lower dry weights than concentration 1/10,000).

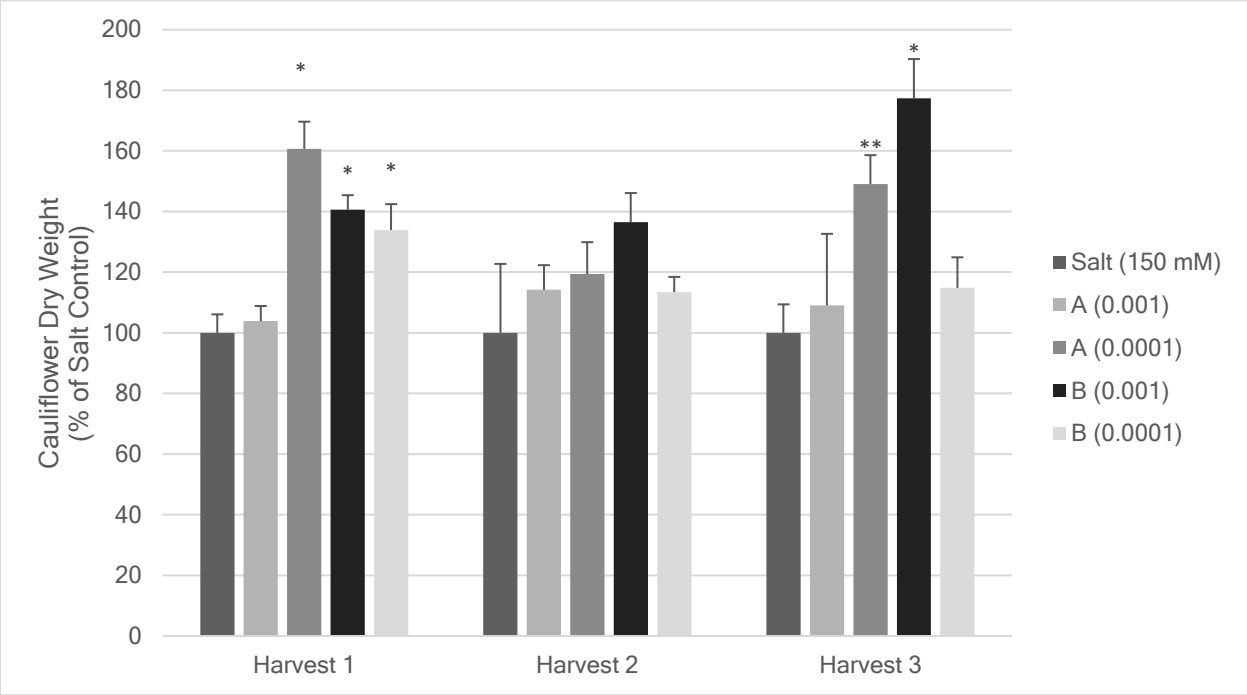

Figure 20. Mean cauliflower leaf dry weight (% of salt control) for three time points, one, two and three weeks post-treatment. Dry weight values were taken from one independent experiment using both Formulations of the product amended with 150 mM salt. One asterisk (*) indicates significant difference from the salt control at $P < 0.01$ and two asterisks (**) indicates significant difference from the salt control at $P < 0.05$ within one time point. Bars indicate standard error.

3.3 Biochemical Analysis

3.3.1 Lipid Peroxidation

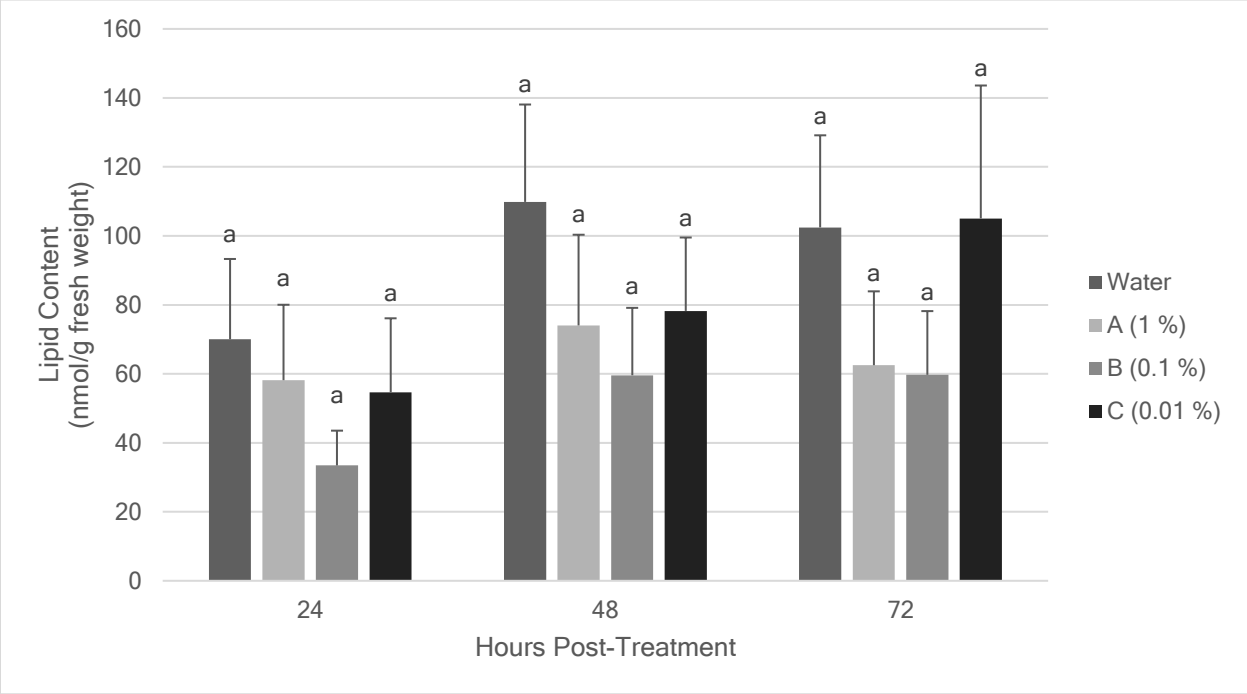

Figure 21. Mean lipid content (nmol g fresh weight^{-1}) for cauliflower. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

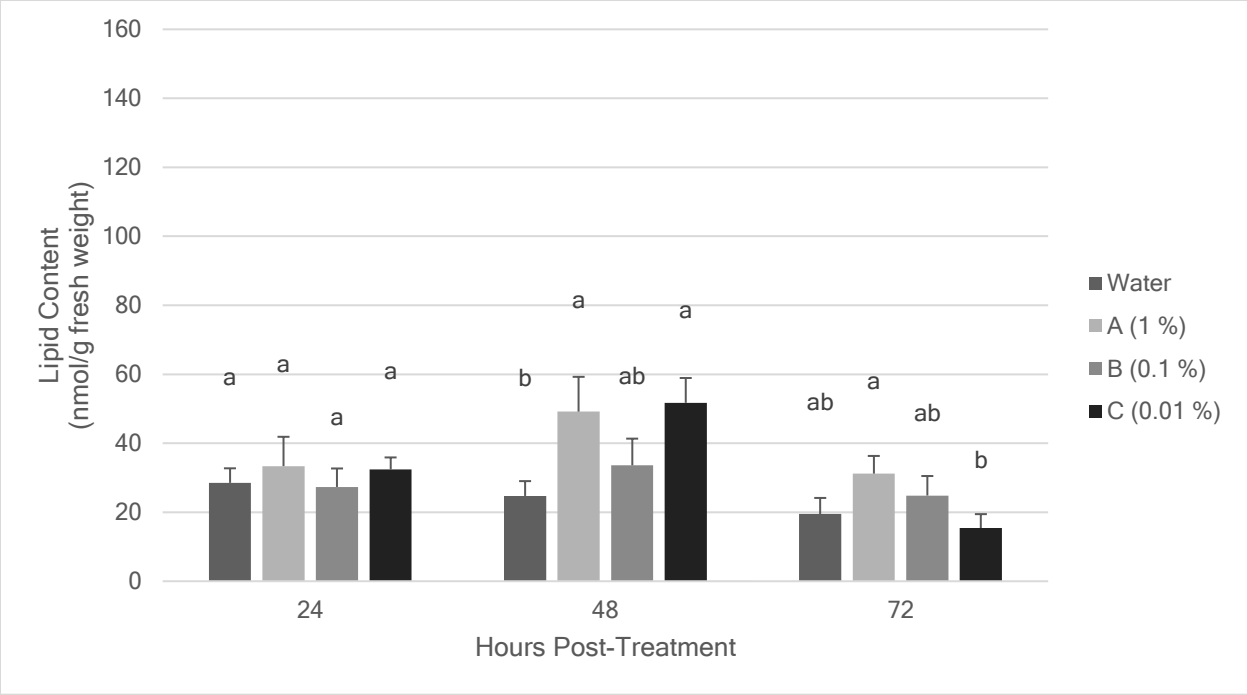

Figure 22. Mean lipid content (nmol g fresh weight^{-1}) for broccoli. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

3.3.2 Total Soluble Sugar Content

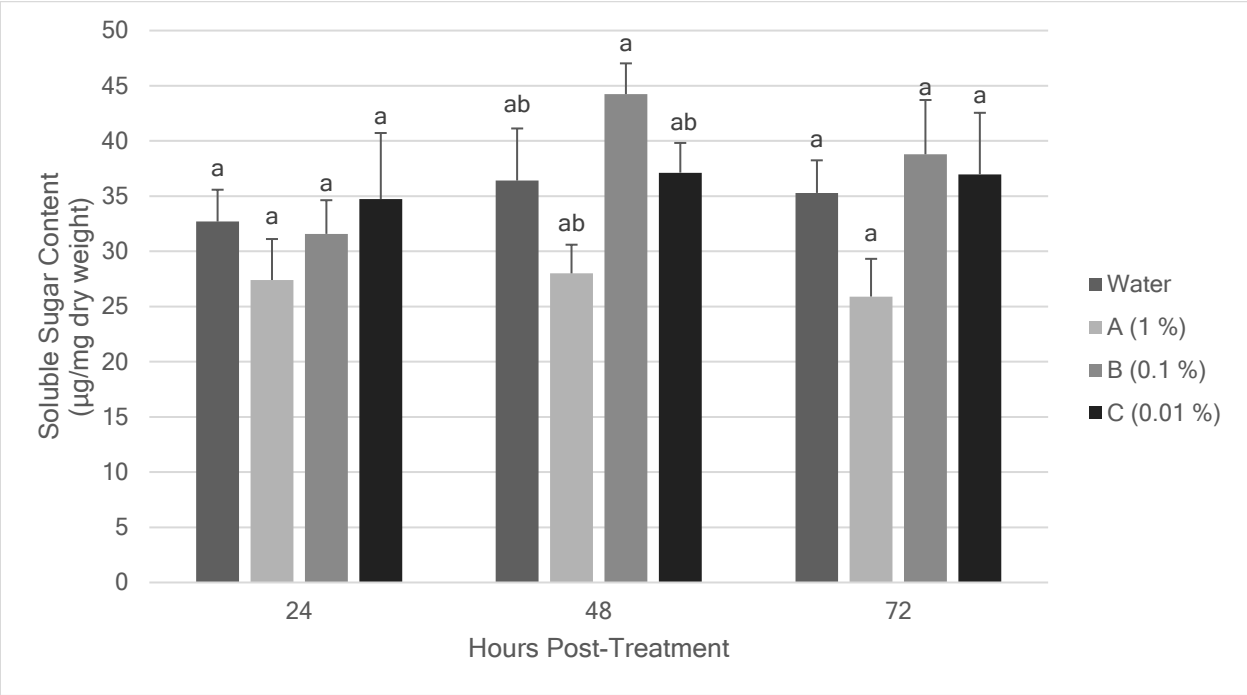

Figure 23. Mean soluble sugar content (µg mg dry weight^{-1}) for cauliflower. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

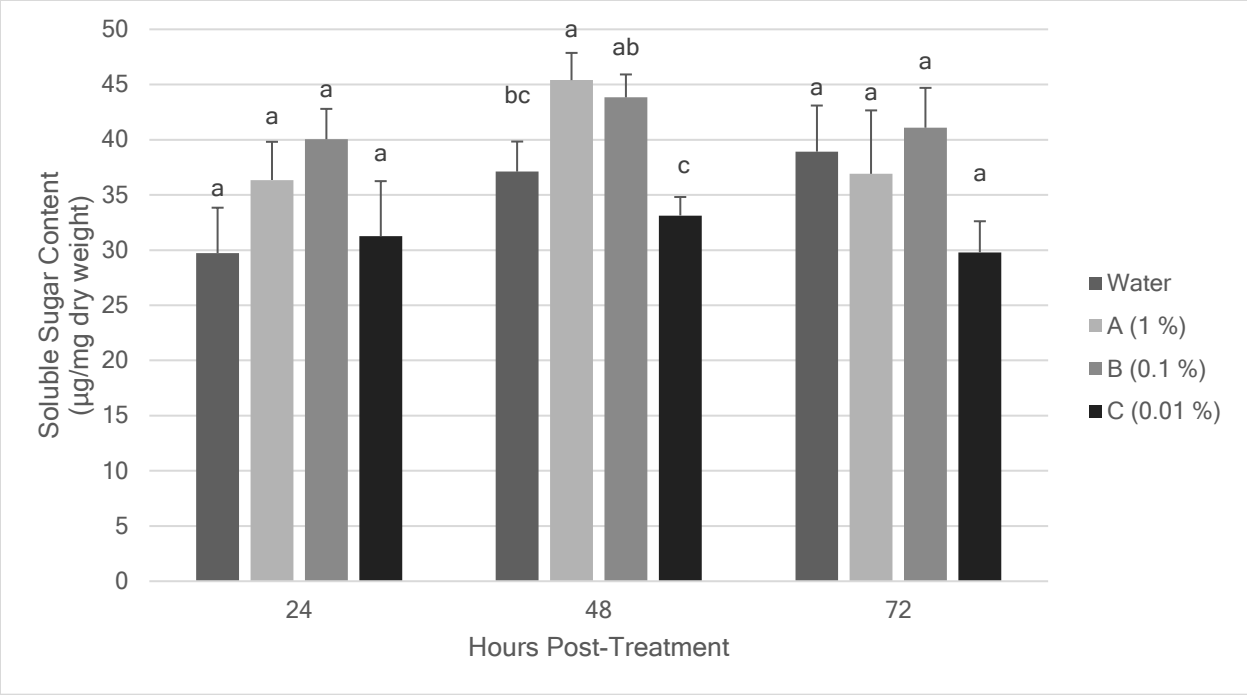

Figure 24. Mean soluble sugar content (µg mg dry weight^{-1}) for broccoli. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

3.3.3 Proline Content

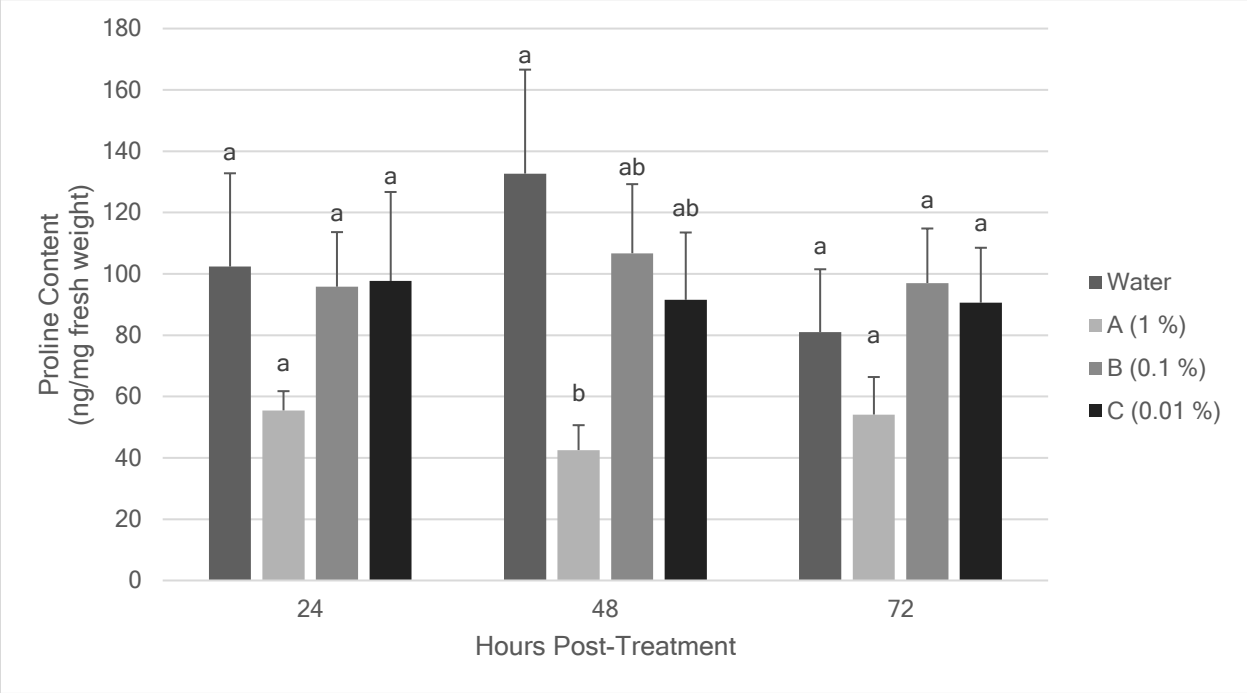

Figure 25. Mean proline content (ng mg fresh weight^{-1}) for cauliflower. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

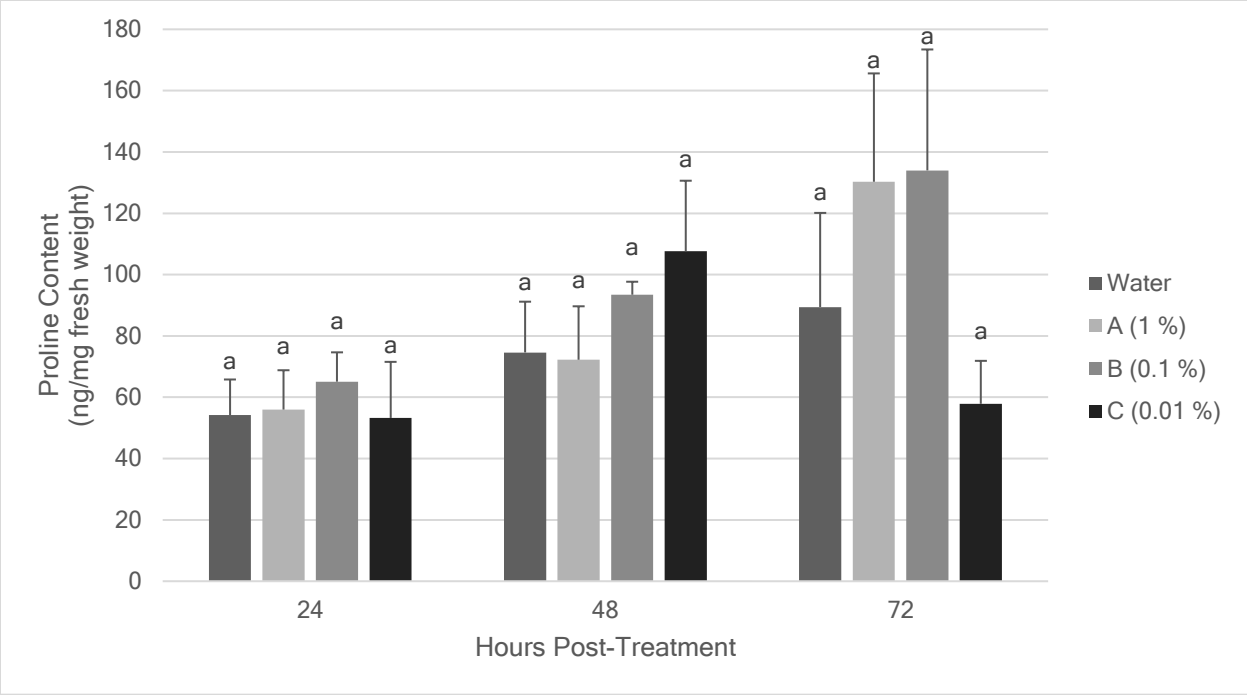

Figure 26. Mean proline content (ng mg fresh weight^{-1}) for broccoli. Values were pooled from two independent experiments. Different letters indicate significant difference at $P < 0.05$ within each time point. Bars indicate standard error.

3.4 Field Research

3.4.1 Effect of OBL product on overall growth and yield of cauliflower.

This experiment was conducted in two unique, commercial field plots (3A and 3B) with varying environmental conditions (Figure 27). The field plot for Experiment 3A was located near the Minas Basin and was widely open to weather conditions such as wind and rain. The field plot for Experiment 3B was further inland, and was less open to weather conditions, as it was almost entirely surrounded by an extensive, mature forested area.

Figure 27. Map showing the two field locations of this field experiment in relation to the farm operation, Melvin Farms Ltd (modified from Google Maps, 2018).

As mentioned in the methodology, the marketable yield was determined by counting the number of marketable cauliflower heads per treatment. In both experiments, the marketable yield was lowest in the plants treated with only water (64 – 70 %) whereas the marketable yield in plants treated with the product was 10 - 15% higher (74 – 90 %) (Figures 28).

In Experiment 3A, all treatment application methods except for the drenching of the lowest concentration (C-DRENCH) resulted in significantly greater marketable yield than the control ($P < 0.05$) (Figure 28). In Experiment 3B, there were no significant differences between treatment application methods (Figure 29).

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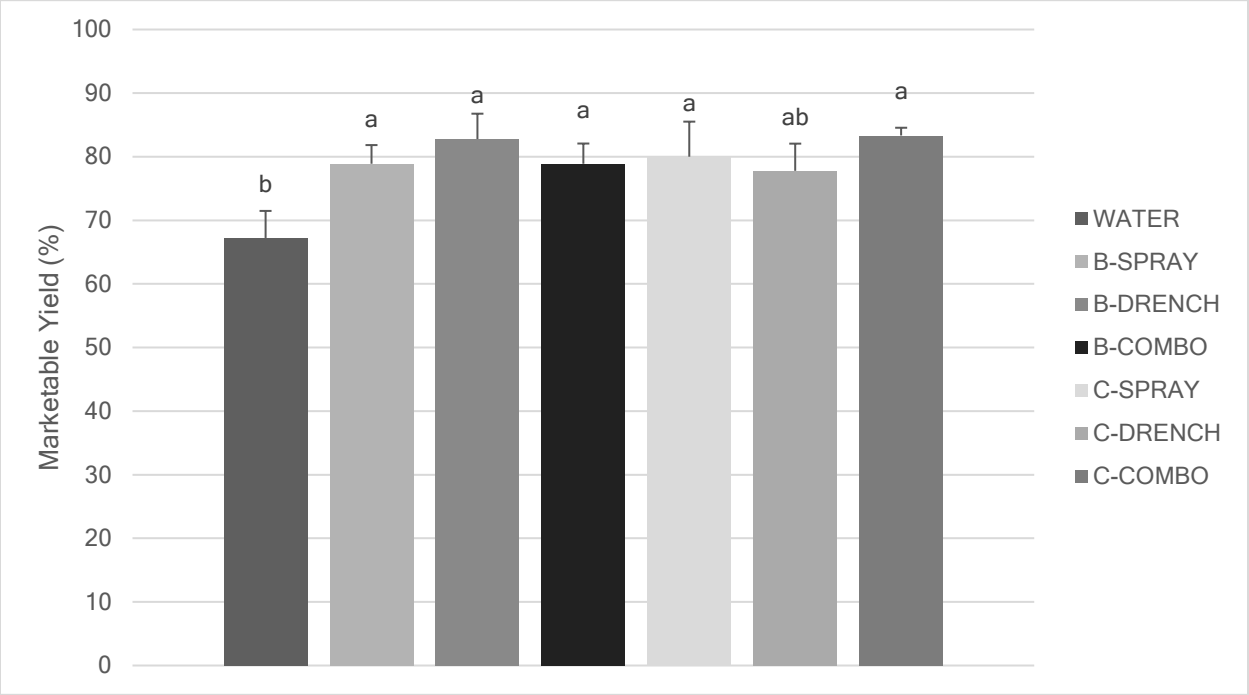

Figure 28. Mean cauliflower marketable yield (%) per treatment application method for field experiment 3A. Different letters indicate significant difference at $P < 0.05$. Bars indicate standard deviation.

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Market Data Forecast. Global Biostimulants Market by Active Ingredients (Fulvic Acid, Protein Hydrolysates, Seaweed Extracts, Amino Acid, Humic Acid, and Others), by Application (Seed, Foliar, Soil), by Crop Type (Fruits & Vegetables, Cereals & Oilseeds, Turfs & Ornaments and Others) and by Region – Global Industry Analysis, Size, Share, Growth, Trends, and Forecasts (2016-2021). Web. www.marketdataforecast.com/market-reports/global-biostimulants-market-1370/ [15 November 2017].

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