AGPATH
Agricultural Pathology and Biological Farming Service
Agricultural Consultants since 1980

PROOF OF EFFICACY. 29/10/2024

REPORT DATE 02/05/25

Pilot trial: comparing water, nutrient solutions with a commercial biological product, Product X, and the same product with the microbes filtered away.

CAVEAT:

This is a one-off trial to determine activity of liquid biological Product X. There were no replications and limited amount of product. The product was taken from an unopened bottle that was returned to the purchaser on request.

Seasol was purchased from Bunnings and sample was taken from an unopened bottle.

This report is not prepared for publication – simply for inclusion on the Agpath website.

NAME OF PRODUCTS: Product X – active microbes; Seasol – nutrient seaweed – no added microbes.

USE OF PRODUCTS: Both liquid products used as a foliar spray to enhance plant growth.

LABEL RATE OF PRODUCT: Product X: 1 in 6 dilution in water across 750sqm (20mL/L); Seasol: 2 – 10mL/L depending on plant. Recommends applying 2 weekly to monthly depending on the plant.

ACTIVE IN PRODUCT: "Special' bacteria! (manufacturer comment).

HYPOTHESIS: *Is it the microbes or the nutrient carrier in commercial products that stimulate growth of plants in the field?*

If it is the nutrients only then why advocate for compost tea over commercial products with 1 to a few microbial species?

REASON: ***Compost tea*** *is made and used fresh with huge diversity of microbes across many functional groups that contribute to nutrient mineralisation in the soil. No additives to keep microbes artificially alive. Compost tea also has nutrients in the carrier solution.*

PRODUCT COST: $1-2/LITRE

COMMERCIAL PRODUCT X*: limited species diversity – loss of viability in container.*

PRODUCT COST: $50/LITRE + GST

Recommended 6 applications across a crop season

SEASOL PRODUCT COST: $11 to $13.75/L for 2L or 20L.

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TRIAL 1:

- FILL TRAYS WITH SAME AMOUNT OF POTTING MIX SO THAT ALL TREATMENTS HAVE SAME BASE. BOILED WATER WAS USED THROUGH THE PREPARATION OF THE PRODUCTS TO MINIMISE ANY CONTAMINATING BACTERIA.
- USE RADISH SEEDS AS FAST-GROWING PLANT EXAMPLE
- PLANT SAME NUMBER OF SEEDS IN EACH TRAY >> 30-32
- TRAY 1 – POTTING MIX + WATER + SEEDS ONLY
- TRAY 2 – POTTING MIX + SEASOL + SEEDS
- TRAY 3 – POTTING MIX + PRODUCT X AT LABEL RATE + SEEDS
- TRAY 4 – POTTING MIX + FILTERED PRODUCT X (0.45UM FILTER) + SEEDS
- PHOITOGRAPH EACH DAY UNTIL HARVEST

1. STREAK 100UL PRODUCT ONTO NUTRIENT AGAR PLATES X 2 – INCUBATE AT 23C
2. STREAK FILTERED PRODUCT ONTO NUTRIENT AGAR PLATES X 2 -INCUBATE AT 23C
3. PHOTOGRAPH COLONY GROWTH
    - APPLY SAME VOLUME OF WATER OR PRODUCT TO APPROPRIATE TRAY
    - PLANT SAME NUMBER OF SEEDS INTO EACH TRAY
    - GROW TRAYS IN GLASS HOUSE AND MONITOR FOR 30 DAYS.
4. HARVEST PLANTS AND MEASURE ROOT LENGTH AND TOPS – WET WEIGHT BIOMASS
5. DRY ROOTS AND TOPS – DRY WEIGHT BIOMASS
6. ASSESS DIFFERENCES BY MEANS AND STANDARD DEVIATIONS
7. WRITE TRIAL AS A FACT SHEET

Methods:

Four trays were set up containing the same amount and consistency of potting mix and compost.
Thirty to thirty-two radish seeds were planted equidistant from each other in the four trays.
Seasol was made up to label rate.
Product X was made up to label rate.
A volume of Product X was filtered through sterile 0.45u filters and made up to label rate.

Nutrient Agar (Amyl Media Pty Ltd) plates were poured and cooled. One hundred microlitres of water, Seasol, Product X and Product X less cells were pipetted onto duplicate plates for each of the four liquids. The drops were spread evenly across the plates using sterile hockey sticks spreaders resulting in an even spread of cells across the agar surface.
These liquids were allowed to dry. The plates were packed up and inverted and incubated at 25C to allow for growth of colonies. First reading was at 24 hours. Readings were taken at 48 and 96 hours.

Results:

Plates were viewed using a stereo microscope at 24, 48 and 96 hours. At 24 hours there were a few mixed colonies on the water plate. On the Seasol plate the colonies were present and mixed. This product is not sterile, so bacterial populations are to be expected. Growth was immediate so it can be deduced that non-spore forming species predominated in the Seasol product. On the

product X plates where cells were concentrated onto a 0.45 u filter, there were no obvious colonies. On the filtered plate there were no colonies.

At 48 hours water and Seasol plates were not visually different from the observations at 24 hours. On the product X plate some bacterial growth was seen growing out from around the filter. The filtered product X plate still showed no growth.

Table 1. Microbial growth on plates each 24 hours from plating.

Hours from plating	Water	Seasol	Product X	Product X less microbes
24 hours	Few mixed colonies	Mixed colonies	0	0
48 hours	Few mixed colonies	Mixed colonies	Minimal growth around filter. Appeared to be single isolate – spore forming rod *Bacillus* sp.	0
96 hours	Few mixed colonies	Mixed colonies	Spread plates shows good growth from 100uL. Appeared to be a single species spore-forming rod *Bacillus* sp.	0

Tray #1 was watered with a volume of clean water only.
Tray #2 was watered with the same volume of Seasol made up to label rate. Seasol is a seaweed nutrient solution with no added microbiology.
Tray #3 was watered with the same volume with Product X at label rate. Product X is a nutrient solution containing live microbes in apparently dormant state.
Tray # 4 was water with the same volume of Product X less the microbial load.

As soon as the radish seedlings had germinated, each tray was foliar sprayed with the same volume of each respective treatment. The radish seedlings were grown on for 30 days before harvest. They were watered each other day from planting to harvest. Raw data table is found at the end of this report.

On harvest day, germinated plants were counted and each plant from each tray was removed carefully and washed clean of any organic matter. Plants from treatments were laid out on paper towel and their total lengths were recoded. See Table 2.

Plants from each treatment were collected into paper bags and their total treatment wet weight recorded. The paper bags were then placed in a 70C oven and 24 hours and then reweighed for dry weight calculation. See Table 2.

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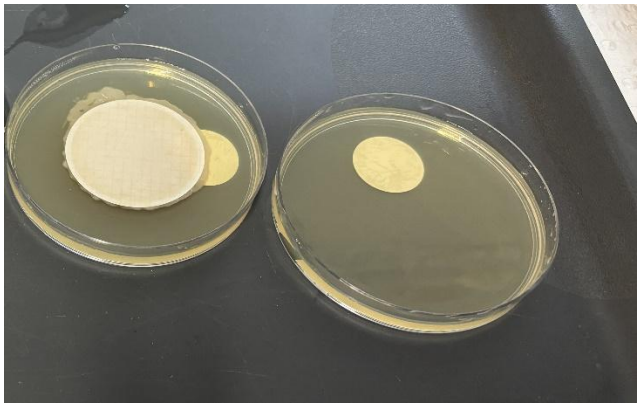
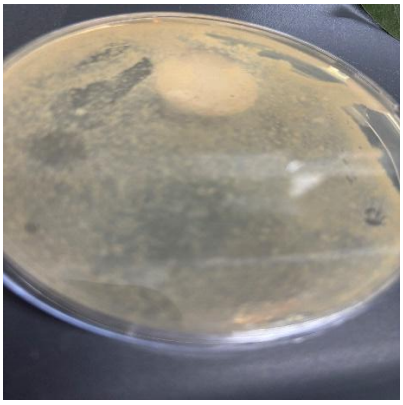
Table 2. Plants germinated. Total length of plantlets; total wet weight of plantlets; total dry weight of plantlets.

Treatments	#plant planted	# plants germinated	Total length of plantlets mm	Mean of lengths	Std Dev of root lengths	Total wet weight gm	Total dry weight gm
Water control	31	31	498	16.06	2.52	20.27	1.32
Seasol	31	31	468	15.1	2.95	29.96	3.56
Product X	31	29	391	13.48	5.05	19.3	2.8
Product X filtered	31	29	437	15.06	3.82	22.49	1.62

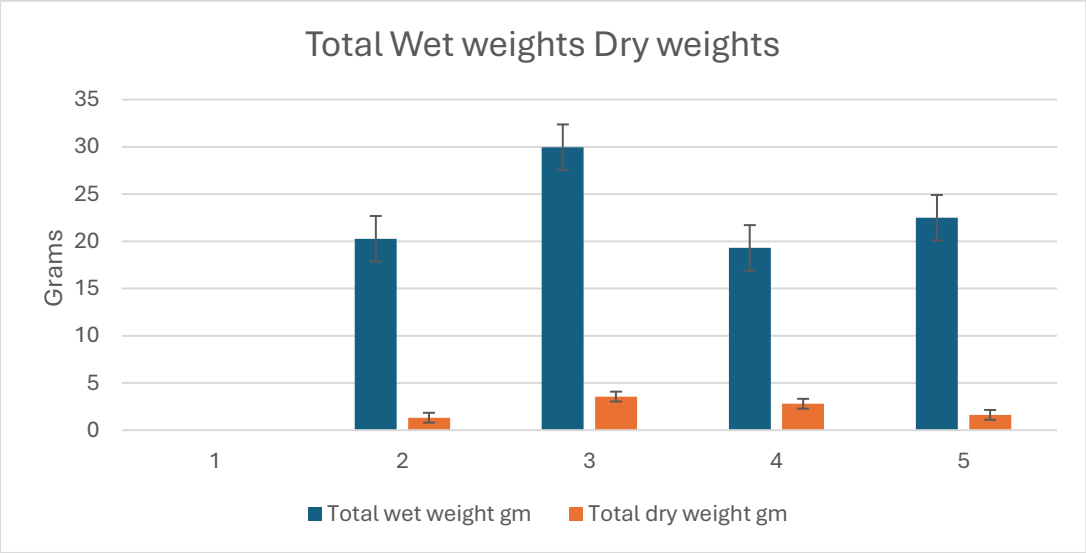
Picture 1. Product x (left) Product X filtered (right) after 48 hours.



Picture 2. Product X streaked onto agar from 48 hour filter plate

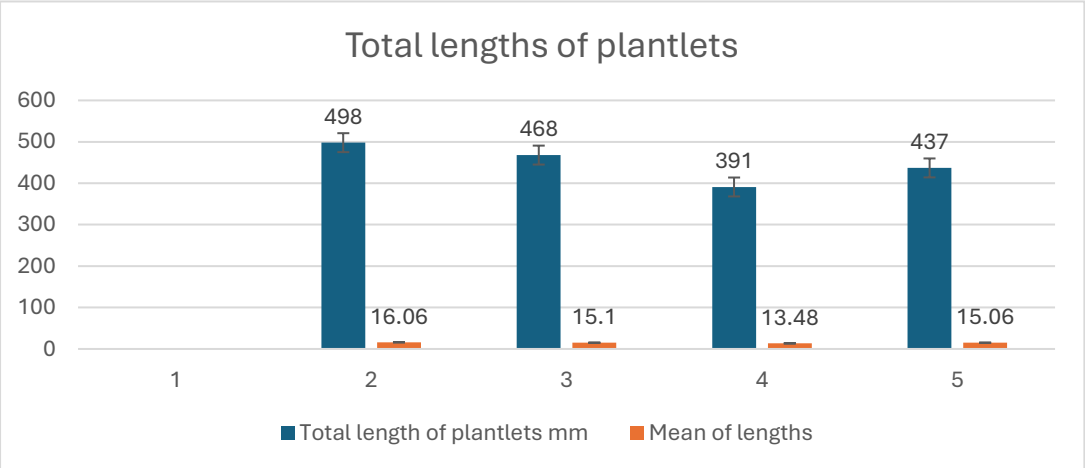


Graph 1. Wet and dry weight data for treatments.



1. Blank
2. Water only
3. Seasol
4. Product X
5. Product X less microbes

Graph 2. Lengths of plantlets.

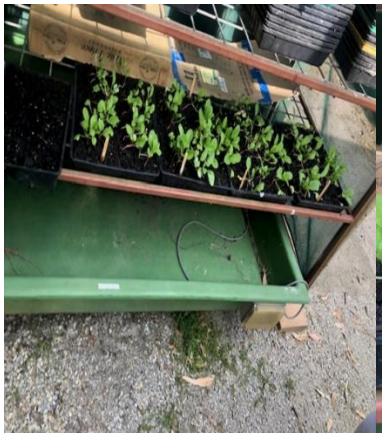


1. Blank
2. Water only
3. Seasol
4. Product X
5. Product X less microbes

Picture 3 Dec 4th 2024



Picture 4 Dec 11th 2024 harvest 22nd Dec



Discussion:

The basis of this small trial was to determine if it is the live and dead bacterial cells that have an influence on plant growth following application of a commercial product, or if it is the nutrient boost given by the carriers in which the cells are presented as the product, that produce the visual effect of growth.

Previous trials on similar products have shown that the presence of bacterial cells did not improve the growth but rather the nutrients increased the nutritional status of the soil or potting mix and stimulated growth of the natural soil or potting mix biota.

No bacterial cells grew on the filtered plates after 96 hours showing that the filtration had removed all bacterial cells (Table 1). No growth was seen on the Product X plates until 36 hours suggesting a lag in activation of cells in the product (Picture 1 & 2). This effect was investigated by making a Gram stain of the culture calls from the 24 & 48-hour plates.

The organism appears to be a spore-forming genus. A *Bacillus* species is the most obvious as this genus is used in many biological commercial products. Spore forming bacteria belong to the prokaryotic Family *Bacillaceae*, a heterogeneous group of rod-shaped, Gram-positive bacteria. Bacterial spores are essentially 'resting structures' in the life cycle of the bacteria, their formation generally resulting from a depletion of some essential nutrient in the growth media, or the accumulation of a signalling compound (quorum sensing) to a critical level (Miller & Bassler, 2001). *Clostridium* and *Bacillus* species are the main genera. *Bacillus* species are identified in part by the position of spore inside the vegetative cell.

In this preliminary study it would be logical to state that the delay in colony growth resulted from a predominance of spores in the product over viable cells – hence the comment by the manufacturer that the cells are 'dormant'. The spores required time to germinate and reproduce in the vegetative state on the nutrient agar. This is what was seen on the plates. This is an Important piece of information for users of the product.

Are users advised to effectively 'resurrect' the product once diluted to label rate before application? If not and the product is applied to plant tissue, then spores may not have conditions in which to germinate and form active vegetative cells. Therefore, potentially, there are no live bacterial cells on the plant surfaces, only nutrient solution and some spores.

The plates carrying the filtered material did not produce any growth over the 96 hours. Filtered substrate was collected for application as a foliar spray onto the growing radish plants in the tray experiment.

Progressive photographs were taken during the growth period (Pictures 3 & 4). Table 2 shows the data from the wet and dry weights, lengths, means and standard deviations.

Graph 1 shows results from each treatment. Growth of plants in Seasol is greater than any of the other treatments. Water alone and the Product X less the cells are the same. Product X is less than all the other treatments. In the dry weight results, water and Product X less the cells are similar. Seasol and Product X are similar.

Graph 2 shows the control plants had best growth, Seasol and product X less cells are similar, and Product X was least effective in growth of plants. Growth in Seasol and Product x less cells is driven by nutrients in the solution and not by microbes.

When considering the wet weights, all treatments gave heavier weights than Product X.

In the dry weights, however, Product X was similar to Seasol and better than water and Product X less the cells (Table 2). This result shows the Product X plants were denser than the plants from the other treatments.

Conclusion:

As with other similar products, it is difficult to justify the cost of the product compared with simple microbial foods such as fish and kelp hydrolysates, low S molasses and rock dust in crop health and growth. Farmers are intelligent but are swayed by hype and convincing presentation from anyone who has a product to sell.

When farmers are shown trials with these products versus trials with simple microbial foods, they understand that they can do better for less cost and improved productivity by staying with readily available, less costly materials. The results for this Product X are similar to those obtained by carrying out the same trials on other products being sold for the same purpose – supposedly to improve plant growth. The buyers of these products need to think through and question the cause and effect of the responses they are observing.

References:

Miller, M.B. & Basslet, B.L. 2001. Quorum sensing in bacteria. Annu Rev Microbio. 2001:55:165-99. doi: 10.1146/annurev.micro.55.1.165.

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RAW DATA.

Product	Date	#seeds planted	Harvest date	#plants germinated	Total length roots to tips	Total wet weight	Total dry weight g
Water control		30	12/12/24	31	498	20.27	1.32
Seasol		30	12/12/24	31	468	29.96	3.56
Product X		30	12/12/24	29	391	19.3	2.8
Product X less microbes		30	12/12/24	29	437	22.49	1.62
	Water control Radish plants wet wt length	Seasol Radish plants wet wt length	Product X Radish plants wet wt length	Product X less microbes Radish plants wet wt length		The nutrient have given more nutrition than nutrients plus bugs.	Season is best
1	15	17	18	17			
2	16	19	17	17			
3	19	14	19	16			
4	13	18	12	14			
5	18	16	18	15			
6	14	15	18	16			

7	16	18	21	10			
8	20	16	17	9			
9	21	16	12	14			
10	17	17	18	5			
11	15	16	17	12			
12	13	18	18	21			
13	18	14	16	18			
14	20	18	16	15			
15	19	18	18	18			
16	18	17	19	15			
17	17	21	14	15			
18	16	14	12	12			
19	18	13	9	18			
20	18	13	13	15			
21	15	15	10	15			
22	13	16	13	16			
23	16	12	8	18			
24	13	16	12	17			
25	12	9	7	15			

26	13	14	6	21			
27	18	9	3	18			
28	14	15	4	19			
29	15	9	6	6			
Total	470	468	391	437			
Mean	16.06	15.09677419	13.48275862	15.06896552			
Standard Deviation	2.52	2.947935308	5.054140379	3.825954918			
	Water	Seasol	Prod X	Prod X less microbes			