Microbial Horticulture: Utilization of Staphylococcus haemolyticus as Plant Growth Promoting Rhizobacteria Soil Drench Inoculum in Cultivating Solanum melongena (Eggplant)

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Abstract

With the emergence of COVID -19 in the Philippines, Filipinos have become accustomed to horticulture (Kampman et al., 2021) and used chemical fertilizers to cultivate their own quality crops, but this material has adverse effects on the environment. The emerging solution for this concern is the inoculation of microorganisms to promote plant growth, called microbial horticulture. Staphylococcus haemolyticus is aerobic bacteria discovered to be a plant growth promoting rhizobacteria in soil through gene testing (Bhattacharyya et al., 2020). Thus, this study utilized a laboratory cultured in Blood Agar Plate media (BAP) Staphylococcus haemolyticus as sole microorganism in soil drenching the Solanum melongena (Eggplant) and measure its daily plant growth in height of plant, length and width of leaves to assess its effects on growth and rate, and evaluate its phenotypic characteristics through destructive analysis in comparison to Agrochemically treated and untreated samples. This research concluded that inoculating laboratory cultured Staphylococcus haemolyticus in Solanum melongena (Eggplant) samples haves shown evident growth in its plant height, length, and width of leaves within three (3) weeks of treatment in an uncontrolled environment than Agro Chemically treated, and untreated plants, but the plant phenotypic parameters treated by all the treatments have no significant difference to each other. Thus, concluding that Staphylococcus haemolyticus has a significant effect on the plant growth, but has no significant effect on the plant phenotype characteristic of Solanum melongena (Eggplant).

Keywords: Agrochemical; Blood Agar Plate (BAP); Inoculation; Microbial horticulture; Plant Cultivation; Plant Growth Rate; Plant phenotype; Rhizobacteria; Soil drenching; Solanum melongena; Staphylococcus

1. Introduction

Horticulture became a custom in every Filipino house and gardens in the emergence of COVID-19 pandemic in 2020 (Kampman et al., 2021). This application of science involves the cultivation of plants in gardens to produce food and raw materials for necessities, comfort, or ornamental purposes to provide for food supply struggles of people during the series of lockdowns. Thus, to improve the growth and yield of the planted crops at home, people began using chemical fertilizers, composts, and pesticides in cultivation. Approximately, more than 50% of the global population feed in a food grown through chemical fertilizers that typically consists of NPK or macronutrients of Nitrogen (N), Phosphorus (P), and Potassium (K) which are also the inorganic chemicals needed by the plants to grow fast. However, the contamination of these environmental bodies led to the pollution in waterways which depletes oxygen levels causing aquatic animals to die. It also contributes to air pollution by too much release of greenhouse gasses to the atmosphere It also damages the leaves of plants causing reduced crop yield, soil acidification that decreases organic matter in topsoil due to excessive Nitrogen, and the mineral depletion that causes less vitamins and minerals to the food

produced in a soil applied with continuous chemical fertilizers (Hunt, 2020). Microbes are recently experimented and tested as an alternative microbiological source of fertilizers to agrochemicals, which might have the potential to reduce the usage of chemical fertilizers in agriculture (Macik et al, 2020). Tea rhizobacteria from Plant growth-promoting properties and antioxidative defense systems were investigated in Darjeeling, India by Bhattacharyya et al., (2020) provided that a nosocomial bacteria, specifically Staphylococcus genera, Sequencing of the Partial 16S rRNA gene was utilized to find it in a rhizosphere of the soil planted with maize and crops which resulted in plant growth promotion because of its significant effect of measurement of total polyphenolics in the leaves of treated rice plants, which is thought to help plants cope with stress and Anti-oxidation provides protection against biotic and abiotic stress and Reactive oxygen species (ROS) deactivation, which if activated can cause damage to lipids, protein, and DNA of plants (Scheiber, et al., 2015). And according to Mollety et al (2021), it produces enzymes such as Auxin indole -3 acetic acid (41µg/ml), solubilized insoluble phosphate, siderophore and ammonia, and synthesizes chitinase enzyme which degrades chitin (Hamid et al., 2013) that contributes to the generation of carbon and nitrogen that is the major component of chlorophyll for converting sunlight energy to produce sugar for plants and building blocks for proteins essential for the life of plants. Without these proteins, the plants wither and die (Eckert, 2020). It also contributes to a considerable amount of Gibberellic acid (7 mg/mL), which is a plant hormone that regulates plant development by encouraging cell division and elongation, affecting the leaves and stems. (Sittig, 2015), and ammonia (903µg/mL) ions responsible for the source of nitrogen that promotes shoot elongation and encourages the seed germination. S. haemolyticus also produces siderophore, and IAA (41µg/ml) production even in a low amount which can regulate cell division, tissue differentiation, elongation, and pathogens. As inoculants, these are used experimentally with plants which can be easily cultivated in a short span of time, such as the Solanacae family that can be grown in pots and at home gardens that may be used as the subject in biological testing, is the Solanum melongena, which is commonly known as Eggplant. (Link, 2017).

Thus, the study's purpose is to utilize the capability of Staphylococcus haemolyticus as an alternative plant growth promoter of Solanum melongena (Eggplant) and evaluate changes in its plant phenotype answering the following specific problems:

1. Is there a significant difference in the growth of plant biometrics (height of plant, length and width of leaves) of the Solanum melongena (EGGPLANT) within 3 weeks (Day 0 - 21) of treatment in a gradual amount of:
a. Staphylococcus haemolyticus soil drench (60 ml)
b. Agrochemical liquid fertilizer (60 ml)
c. Distilled water
2. Is there a significant difference in the percentage growth rate of plant (height, length and width) of the Solanum melongena (EGGPLANT) treated with Staphylococcus haemolyticus soil drench, Agrochemical liquid fertilizer, and Distilled water?
3. Is there a significant difference in the plant phenotype (destructive analysis) of the Solanum melongena (EGGPLANT) treated with Staphylococcus haemolyticus soil drench, Agrochemical liquid fertilizer, and Distilled water?
a. Fresh weight
b. Root length density (RLD)

2. Methodology

2.1 Research design

This study used the experimental research to investigate the effect of treating Solanum melongena (Eggplant) with Staphylococcus haemolyticus as plant growth promoting soil drench inoculum by analyzing

its rate of growth and plant phenotype. Experimental research was utilized to compare the variables Solanum melongena (Eggplant) treated with Treatment A (Staphylococcus haemolyticus), Treatment B (Agrochemical liquid fertilizer) and Distilled Water. These treatments and controls were manipulated by the researchers to give no or expected result. This was used to identify the effectiveness of Staphylococcus haemolyticus as plant growth promoting rhizobacteria soil drench inoculum compared with other treatments on the improvement of the plant in uncontrolled environment.

2.2 Locale of the study

The known cultured bacteria Staphylococcus haemolyticus was acquired at Marikina Valley Medical Center in a Brain Heart Infusion (BHI) and Blood Agar Plate (BAP) medium, and undergone the laboratory process of dilution to create a soil drench solution of 10:1000 in NSS at PHINMA St. Jude college laboratory, and the soil drenching process and data gathering was done in an uncontrolled environment.

2.3 Sample of the study

This study used Solanum melongena (Eggplant) seeds with an Open Pollinated variety of Eggplant Long Purple, Philippine Seed Industry Association (PSIA) certified. The plants are pre-planted for one (1) month in pots before the introduction of the treatments as soil drench solutions. All the treatments have three (3) corresponding plant replicates, with a total of nine (9) plants in the experiment, and all the plant samples are randomly arranged at one exact location in the experimental field, and are soil drenched in a gradual amount of (week 1: 500 ml, week2: 1000 ml, week3: 1500 ml).

2.4 Data gathering and procedures

In the application of treatments, the height of the plant, length and width of the leaf, and temperature was recorded every day for three weeks. The height of the plant is measured using tape measure, from the top of soil to the top of the plant (suggested by the Bureau of Plant Industry guidelines). In measuring the plant phenotype, Fresh weight is measured by weighing the pot including the plant and after harvesting the plant with a formula of Fresh weight = [weight of pot before cutting (pot+plant)]-weight after (pot). The formula used to measure the phenotype of the plant was derived from the European Plant Phenotyping Network. In Root Length Density (RLD), tape measure was used to obtain the root length, RLD measures the total length of roots per unit of volume of soil with a formula of Root length density (RLD) = root length / soil volume (cm cm-3). To solve for the soil volume needed in the RLD, the base side (a) and top side (b) of the pot and height of the soil was measured using a ruler and calculated using the formula of volume truncated square pyramid from Reisan Casio v=13(a2+ab+b2) h. The validation of measurement was signed by the research adviser and microbiologist.

2.5 Data analysis

The plant growth rate is calculated using a Straight line percentage change method on a weekly basis (1-7 days) in 3 weeks, which uses a formula of PR= [(Vf - Vi)/Vi]×100/N, whereas, PR is the percentage rate of growth of plant, Vf as the last day of treatment (Day 21), Vi as the 1'st day of treatment (Day 1), and N as the period of time. Two-way Analysis of Variance (ANOVA) was used to determine the growth in cm from Day 0 to Day 21 in the statistical analysis of the data. To confirm the significance of the data, Bonferroni post

hoc test was used. A 95 percent confidence level and a threshold of significance of 0.05 were employed in the statistical analysis of p-value.

2. Results and Discussion

3.1Plant Growth

In the plant growth of Solanum melongena (Eggplant), the researchers measured the plant samples every day from initial day until the final day of treatment every 7:30 am using measuring materials and recorded the raw data in centimeters, and undergone a statistical analysis to know if there is a significant difference in the growth of plants within 21 days of treatment.

Legend: ns: Not Significant, * and **: Significant, ***: Extremely Significant

Table 1. Plant growth in height of Solanum melongena (Eggplant) within 21 days of treatment

Source of Variance (Days of treatment)	% of total variance	P-value	Summary
Treatment A	93.62	<0.0001	***
Treatment B	1.53	1	ns
D.H20	46.55	<0.0001	***

On the plant growth in height of Solanum melongena (Eggplant), Treatment A and Distilled water has extremely significant difference from Day 0 to Day 21 of treatment with a P – value of <0.0001, while Treatment B has no significant difference in growth with a P- value of 1.

Table 2. Plant growth in length of leaves of Solanum melongena (Eggplant) within 21 days of treatment

Source of Variance (Days of treatment)	% of total variance	P-value	Summary
Treatment A	90	<0.0001	***
Treatment B	5.26	0.7898	ns
D.H20	46.32	<0.0001	***

On the plant growth in length of leaves of Solanum melongena (Eggplant), Treatment A and Distilled water has extremely significant difference from Day 0 to Day 21 of treatment with a P – value of <0.0001, while Treatment B has no significant difference in growth with a P- value of >0.7898.

Table 3. Plant growth in width of leaves of Solanum melongena (Eggplant) within 21 days of treatment

Source of Variance (Days of treatment)	% of total variance	P-value	Summary
Treatment A	60.71	<0.0001	***
Treatment B	11.13	0.2017	ns
D.H20	48.44	<0.0001	***

On the plant growth in width of leaves of Solanum melongena (Eggplant), Treatment A and Distilled water has extremely significant difference from Day 0 to Day 21 of treatment with a P – value of <0.0001, while Treatment B has no significant difference in growth with a P- value of 0.2017

3.2 Percentage Growth Rate

In this category, researchers aim to know if there is a significant difference between the percentage growth rate of each treatment by using the straight-line percentage change method and evaluate the difference through One-way ANOVA.

Table 4. Percentage Growth Rate in height of Solanum melongena (Eggplant) between treatments

	Mean Difference	t	P-value	Summary
Treatment A vs Treatment B	310	5.5	0.0035	**
Treatment A vs D.H20	240	4.3	<0.05	*
Treatment B vs D.H20	-68	1.2	>0.05	ns

Treatment A has significant difference in growth rate of height of Solanum melongena (Eggplant) from Treatment B with a P value of 0.0035. Treatment A also has significant difference from Distilled water with a P value of <0.05, while Treatment B and Distilled water has no significant difference in percentage growth rate with P value of >0.05.

Table 5. Percentage Growth Rate in length of leaves of Solanum melongena (Eggplant) between treatments

	Mean Difference	t	P-value	Summary
Treatment A vs Treatment B	250	6.3	0.0019	**
Treatment A vs D.H20	170	4.4	<0.05	*
Treatment B vs D.H20	-74	1.9	>0.05	ns

Treatment A has significant difference in growth rate of length of leaves of Solanum melongena (Eggplant) from Treatment B with a P value of 0.0019. Treatment A also has significant difference from Distilled water with a P value of <0.05, while Treatment B and Distilled water has no significant difference in percentage

growth rate with P value of >0.05.

Table 6. Percentage Growth Rate in length of leaves of Solanum melongena (Eggplant) between treatments

	Mean Difference	t	P-value	Summary
Treatment A vs Treatment B	230	5.3	0.0052	**
Treatment A vs D.H20	150	3.4	<0.05	*
Treatment B vs D.H20	-82	1.8	>0.05	ns

Treatment A has significant difference in growth rate of width of leaves of Solanum melongena (Eggplant) from Treatment B with a P value of 0.0052. Treatment A also has significant difference from Distilled water with a P value of <0.05, while Treatment B and Distilled water has no significant difference in percentage growth rate with P value of >0.05.

3.3 Plant phenotyping

The plant phenotyping of plant samples includes the evaluation of Fresh weight and Root length density in destructive analysis and calculation and differentiated through one-way ANOVA in calculating the significant difference of the treatments.

Table 7. Plant phenotyping in Fresh weight of Solanum melongena (Eggplant) between treatments

	Mean Difference	t	P-value	Summary
Treatment A vs Treatment B	-45	0.86	>0.05	ns
Treatment A vs D.H20	-3.3	0.064	>0.05	ns
Treatment B vs D.H20	42	0.8	>0.05	ns

Treatment A, Treatment B, and Distilled water treated Solanum melongena (Eggplant) have no significant difference in the fresh weight with a P value of >0.05.

Table 8. Plant phenotyping in Root Length Density of Solanum melongena (Eggplant) between treatments

	Mean Difference	t	P-value	Summary
Treatment A vs Treatment B	0.0014	2.8	>0.05	ns
Treatment A vs D.H20	0.00086	1.7	>0.05	ns
Treatment B vs D.H20	-0.00055	1.1	>0.05	ns

Treatment A, Treatment B, and Distilled water treated Solanum melongena (Eggplant) have no significant difference in the root length density with a P value of >0.05.

4. Conclusions and Recommendation

The following conclusions were formed by the researcher based on the study's findings: Staphylococcus haemolyticus soil drench inoculum and Distilled water have significant effect in the growth of Solanum melongena (Eggplant) throughout the 21 days of cultivation in an uncontrolled environment. Staphylococcus haemolyticus soil drench inoculum treatment has a significant difference in percentage plant growth rate of Solanum melongena (Eggplant) between Agrochemical and Distilled water. Staphylococcus haemolyticus soil drench inoculum treatment has no significant effect in plant phenotypic parameters of Solanum melongena (Eggplant).

The researchers recommend based on these conclusions are Researchers should make a nutritional media for the Staphylococcus haemolyticus to sustain its lifespan in the soil drench solution, and utilize the capability of other BSL 1 microorganisms (E.coli, Agrobacterium radiobacteria, Aspergillus niger, Bacillus thuringiensis, Micrococcus luteus, Neurospora crassa, Serratia marcescens) in plant growth promotion, and aside from plant growth, and phenotype, they may also evaluate the maturation of Solanum melongena (Eggplant) (flowering, fruit-bearing) in a controlled environment to test if the crop is edible and if the bacteria has effects to the fruit's cellular structures.

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