# The Effect of Whey, *Azadirachta indica*, and Powdered Whole Milk on *Monilinia fructicola*

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Throughout the United States of America, stone fruits like nectarine, peach, apricot, are an important component of the fruit economy, particularly in South Carolina and Georgia. Unfortunately, during pre-harvest, harvest, and post-harvest seasons, these fruits frequently contract *Monilinia fructicola* (brown rot producing fungus). A potential method of eradicating *Monilinia fructicola* was proposed using whey, powdered whole milk, *Azadirachta indica* oil, and a combination of all three substances. It was hypothesized, if the 2% concentration of the combined mixture of solutes is added to the *Monilinia fructicola,* then the number of fungal spores will decrease the most due to the mixture having the beneficial characteristics of all 3 substances, thus eliminating the fungi most effectively. The fungus was cultured on petri dishes for 5-9 days. After, the cultures were sub-cultured into the remaining petri dishes. 9 days later, the fungal spores were counted using a microscope, hemocytometer, and fungi counter. Data were recorded, and 2% concentrations (v/v, w/v) of the treatments were created. After 9 days, the treatments were applied, and data were collected again. Statistically significant results ($t(29)=17.6244$, $p < 0.001$; $t(29)=18.9747$, $p <0.001$); $t(29)=22.9349$, $p < 0.001$; $t(29)=28.9039$, $p < 0.001$) were found between the following groups (before and after): control, whey, powdered whole milk, *Azadirachta indica* oil, and combination. The combination inhibited the growth of the fungi most significantly ($t(29)=28.9039$, $p < 0.001$) (Before: M=430600000, SD=80565501.3; After: M=6600000, SD=5035049.57) supporting the hypothesis.

## Introduction

Currently, the yield of stone fruits (peaches, apricots, nectarines, plums, lychees, and mangoes) has decreased greatly due to the brown rot fungus, *Monilinia fructicola*, bruising, wounding, and rotting them[1]. Because of this issue, the farmers who cultivate stone fruits are being negatively impacted due to the reduction in the number of healthy stone fruits, especially peaches, available for marketing. Marketing of these fruits is most important in South Carolina, Georgia, and California as these are the top three peach-producing states. Currently, only 2.6 million bushels of peaches are produced annually, whereas previously (1851-1928), 8 million bushels were produced each year[2]. *M. fructicola* is an invasive fungus which can affect other fruit located near the infected fruit. Based on Beckerman's research, unripe fruit can resist brown rot, however this fruit can still be infected through wounds created on the fruit by animals or detritus[1]. Additionally, during the wet season, orchards are known to suffer 75% reduction of crops due to brown rot[3]. This fungus has caused cherry production in some areas impossible because the brown rot cannot be controlled[4]. Not only this, *M. fructicola* has spread to many areas in the world including India, New Zealand, Japan, Oceania, South America, and many more[4].

The three types of substances which were used in this study include *Azadirachta indica*, whey, and powdered whole milk. Neem oil (*Azadirachta indica)* consists of over 100 biologically active compounds including azadirachtin, the most important component[5]. This oil is one of the least toxic oils to humans and shows low toxicity to other organisms. Neem oil has been used as an insecticide as well[5]. This oil can also help reduce pests in agriculture and act as a fertilizer to enhance the quality of crops, potentially including peaches and other stone fruits. Another substance which has the potential to destroy brown rot on peaches and other stone fruits is whey, which are the loose fluids of milk that are created after curd is formed[6]. According to Augustin (2003), the amount of micronutrients like potassium, calcium, phosphorus and zinc in whey are discussed which are vital minerals for the human body (healthy for humans to consume)[6]. Specifically, liquid whey is high in calories, carbohydrates, proteins, vitamins, and minerals. However, based on Iannotti, whey can be harmful for the environment due to its acidity and amount of fat[7]. On the other hand, because of whey's acidity, the brown rot fungus can potentially reduce. Powdered whole milk was used because it is the cheapest source of dairy foods, and whole milk has a higher amount of fat content rather than skim or another type of milk. Additionally, based on Marie Iannotti (2019), a horticulture educator, states that one main reason powdered milk is used is because it can help prevent the production of fungi on trees due to the proteins in milk conveying an antiseptic-like effect when exposed to sunlight[7]. Four treatment solutions were made and tested: 2% of solutes of whey (v/v), 2% of solutes of powdered whole milk (w/v), 2% of solutes of neem oil (v/v), and 2% of solutes of a combination of the substances (v/v).

The number of fungal spores of *Monilinia fructicola* present were measured before and after treatment with the solutions (whey, *Azadirachta indica*, and powdered whole milk). Calculating the change in the fungal spores determined how effective each substance was. The fungi formed on the petri dishes were counted using a hemocytometer and a microscope to identify the number of spores present. During the counting process, differences between the physical appearance of the spores before and after were compared. The color of the fungi slightly darkened after the treatments were added.

Previous research examined the fungal postharvest decay of apples, oranges, bananas, mangoes, and grapefruits sold in local markets in Sana'a city, Yemen[8]. Many fungi were isolated and identified such as *Penicillium expansum* located in the apple, and *Aspergillus niger*, located in the mango. Due to diseases caused by these fungal pathogens, such as brown rot and other fungal postharvest decay, losses of these agricultural crops occur every year[8]. One primary example of postharvest decay is the spoilage of the fruit. Because of spoilage, the condition of fruits (peaches, plums, apples, etc.) are less palatable and more pernicious, causing changes in taste, smell, and appearance which results in losses in the agricultural economy[8]. Furthermore, another study showed different methods of inhibiting the growth of *Monilinia fructicola* on stone fruits including peaches and nectarines[9]. In the study, hydrocooling (adding fruits to cold water or vaccum cooling), storing fruits in a cold room (room with low temperature), water dump (method for removing fruits from harvest bins), sorting (eliminates decaying fruits), and a cooling tunnel (removal of field heat after the harvest of fruit) were methods tested to reduce *Monilinia fructicola* in peaches and nectarines. However, most of these cooling methods were ineffective on the peaches and nectarines apart from hydrocooling and water dump methods, which slightly decreased *Monilinia fructicola* present on the nectarines[9]. Additionally, according to Crisp and his colleagues, whey and milk eliminated the fungi present on the grape vines due to the fatty acids and free radicals present in these substances[10]. Based on the results of this previous study, whey and milk could potentially reduce the fungus, *Monilinia fructicola.* Furthermore, it was proven that neem oil reduced the fungi, *Fusarium sp., Rhizopus sp., Curvularia sp., and Aspergillus sp*[11]. Based on the results of this study, neem oil could also possibly reduce *Monilinia fructicola*.

The purpose of this experiment was to eliminate the fungus causing the brown rot present on stone fruit trees by using whey, *Azadirachta indica*, powdered milk, and a combination of the three. If successful, these substances could potentially be used to increase the quantity of robust fruits produced, resulting in profit, particularly in states such as South Carolina, California, and Georgia. It was hypothesized, if the 2% concentration of the combined mixture of the 3 solutes (whey (v/v), *Azadirachta indica* (v/v), and powdered whole milk (w/v)) were added to the *Monilinia fructicola*, then the number of fungi spores would decrease the most because the mixture contains the beneficial characteristics of the 3 substances, thus eliminating the fungi most effectively. *Monilinia fructicola* (from infected fruits) was placed on a petri dish with potato dextrose agar and incubated for 9 days at 25 °C as a source to produce fungi spores. After that, the solutions of whey, *Azadirachta indica*, and powdered whole milk (no solution, 2% of each substance, 2% of combination of substances) were added to the petri dishes which were then incubated for another 9 days at 25 °C. Then, the number of fungi spores were counted for each solution using a hemocytometer and a microscope.

## Methods

Personal protective equipment was worn throughout experimentation. The petri dishes were labeled, corresponding to the amount and type of solution. To prepare agar for the petri dishes, 100.0 grams of potato dextrose agar powder and 2.5 L of distilled water were added to a bowl. Then, the solution was boiled on a hot plate and mixed well with a magnetic stirrer. The media was sterilized by autoclaving at 121°C for 30 minutes[12]. After cooling, the agar was poured into 82 sterile petri dishes. Using scalpel and forceps, portions of mummified peach (Blythewood Farmers Market) were cut and placed on 7 petri dishes[13]. The dishes were placed in an incubator at 25 °C for 9 days to culture the fungi. After the culture was formed, using an inoculating loop, small pieces of the culture were placed on 75 petri dishes with agar. The dishes were placed in the incubator at 25 °C for 9 days. After incubation, the fungal colonies were counted and recorded using a hemocytometer. To use the hemocytometer, a spore suspension was created using the mycelium from the petri dishes, Polysorbate 80, and distilled water. First, half of the fungal colonies in each petri dish were removed using a surgical blade and placed on a watch glass. Then, 9 mL of water and 1 drop of Polysorbate 80 (Tween 80) were added on 0.1 mL of the mycelium on the watch glass to produce a spore suspension. Following this, the suspension was strained using a cheesecloth.

To determine the spore concentration, 15µL of the spore suspension was added to the grooves of the hemocytometer using a micropipette as shown in figure 1, the cover slide was then placed over the hemocytometer. The hemocytometer was placed under the microscope and the number of spores were counted only in the 4 corner squares (16 squares each). The average of the number of spores in the 4 squares were calculated. Then, the total spore count was calculated using the equation: spores/mL = (n) x $10^4$ x 100 (dilution factor), (n is equal to the average spore count per square) [15]. This was repeated for all the petri dishes.

Four types of treatment solutions of whey, *Azadirachta indica*, powdered whole milk, and a combination of the 3 substances (equal quantities mixed together) were prepared. To make the treatment solutions, two mL or grams of each treatment was taken and added with 98 mL of water to produce a diluted solution. The dilution factor was calculated by dividing 2 by 98 to produce a quotient of 0.02 (add 2 mL with the 98 mL). Then, the percentage of each solute was calculated by multiplying the dilution factor with 100 thus giving a product of 2% (refer to experimental design diagram). The solutions were added to each petri dish corresponding to the appropriate solute with a pipet. Then, the 75 petri dishes were placed in the incubator for another 9 days at 25 degrees Celsius. Lastly, the number of fungi colonies remaining for each type of solute was recorded using the same method above for the spore count. The culturing of fungi and the addition of the solutes were repeated 1 more time with the 75 petri dishes for a total of 30 trials. To dispose of the fungi, a 20% bleach solution and 70% isopropyl alcohol were added to the petri dishes and allowed to stand for 24 hours to ensure the culture did not form again.

## Results

Five different treatments were performed on the fungi, *Monilinia fructicola*, including the control, whey, powdered whole milk, neem oil, and a combination of all three. Throughout the experiment, physical differences between the fungal growth for each treatment were shown. Color change from white, yellow, to green was present in the petri dishes for the treatments. Not only this, physically it could be seen that the number of spores greatly decreased than the "before" group.

Based on the data present in table 1, a significant decrease is present among all the five treatments, except the control group. According to the summary data table #1 (raw table located in Appendix A), the mean of the difference among the control group is 16633333.33 showing an increase in the amount of fungi. The mean difference for the powdered whole milk is -414300000 showing a significant difference between the before and after groups meaning that less fungi was present after. The mean difference for the whey treatment was -414666666.67 showing a significant difference as well. Continued, the mean difference for the *Azadirachta indica* oil was -378566666.67 which showed a significant decrease, but not as large as the powdered whole milk nor the whey. Lastly, the combination produced a mean difference of -424000000.00 which is the highest value out of all the treatments. Furthermore, for each treatment, a single paired-t test was done to compare the before and after. Table 2 presents a summary table for the control values. According to the trial for control, statistically significant results were found at $t(29) = 5.7052$, $p < 0.001$. Also, for this specific data set, the mean, standard deviation, and SE mean increased higher in the "after" group meaning more fungi was produced. Table 3 shows a summary table for whey. For the trial of whey, another single paired-t test was done to compare the before and after. The statistically significant results, $t(29) = 17.6244$, $p < 0.001$, were found based on the data. Table 4 shows a summary table for the treatment, powdered whole milk. A single paired-t test was done again to compare the before and after of the powdered milk. For the trial for powdered whole milk, the data was found to be statistically significant as the results were found to be $t(29) = 18.947$, $p <0.001$. Table 5 shows the summary table for neem oil. According to table #5, the results can be proven statistically significant, $t(29) = 22.9349$, $p<0.001$. Table 6 presents a summary table for the combination treatment. Based on table #6, the results are statistically significant, $t(29)=28.9039$, $p<0.001$. Not only this, because the t value of the combination treatment is the largest out of all the other treatments, this provides greater evidence of a larger significant difference. Also, the mean decreased from 430600000.00 to 6600000.00 which is the highest difference among all the treatments.

Figure 2 represents the effect of each treatment on the fungi spore count. Based on the data obtained, the number of fungal spores greatly decreased. The combination treatment was the most effective at decreasing the number of spores. As expected, the control group did not suppress the fungal spore count. Figure 3 shows the effect of the differences between the before and after of each treatment on the number of spores decreased. Based on the box plot, the combination can be seen to have the least range, while the whey had the highest range which shows the difference in variation. Not only this, the number of spores that appear in the petri dish had reduced for all the treatments except the control.

## Discussion

Whey, *Azadirachta indica*, powdered milk, and a combination of all three were tested to determine their effectiveness in eliminating *Monilinia* *fructicola*. The study's importance was to determine a method to increase the quantity of healthy fruits being sold into the markets, resulting in a higher amount of profit of stone fruits, particularly in areas like the South, such as South Carolina and Georgia. Whey, which contains many micronutrients, demonstrated the ability to decrease the brown rot fungus. *Azadirachta indica* oil was used because this helps reduce pests in agriculture and acts as a fertilizer to enhance the quality of crops. Powdered whole milk are filled with many minerals and it can help prevent the production of fungi due to the proteins in milk, which offer an antiseptic-like effect when exposed to sunlight (Iannotti, M., 2019). Previous results showed a decrease in mildew when treated with milk and whey (Crisp, 2006). Not only this, another study showed different methods including hydrocooling, storing fruits in a cold room, water dump, and cooling tunnel would decrease *Monilinia fructicola* in nectarines and peaches, but it was not very effective (Bernat, M., 2017). Based on these results, it was hypothesized, if the 2% concentration of the combined mixture of solutes (whey (v/v), *Azadirachta indica* (v/v), and powdered whole milk (w/v)) is added to *Monilinia fructicola*, then the number of fungi spores will decrease the most because the mixture contains the beneficial characteristics of all three substances, and thus eliminating the fungi most effectively.

The combination of all 3 substances produced the largest decrease in spore count. This shows that the combination had the greatest difference between the before and after out of all 5 treatments. According to the data, the mean difference for the combination before and after were the highest, being 430600000.00 spores/mL and 6600000.00 spores/mL. Not only this, the largest t-value was produced by the combination of 28.9309. Based on the results, it can be concluded that the hypothesis was supported.

Counting the fungal spores throughout the experiment may not have been as accurate as possible. With the use of a scanning electron microscope and Image J, the results could produce more precise values, but this equipment was unavailable. Instead, a hemocytometer was used to count the fungi, as an alternative which provided the necessary results. Additionally, allowing the fungi to grow for a longer period of time could possibly alter the results.

For future research, working with fungi: *Monilinia fructicola, Monilinia laxa, and Monilinia fructigena* will help reduce or eliminate a wider range of fruit such as apple, pear, and other pome fruits. With all 3 species eradicated, more fruits will be produced to increase profit in the agricultural economy. To reduce the fungi, sodium bicarbonate and *Taraxacum officinale* could be used due to their beneficial properties to humans, plants, and animals. For example, sodium bicarbonate is an antifungal agent known to destroy other species of fungi and shown to reduce powdery mildew and black spots in fruits and trees[16]. Additionally, sodium bicarbonate is not toxic to animals, is inexpensive, and is readily available. *Taraxacum officinale's* hollow stem has milky sap which has antifungal properties, germicidal, and insecticidal properties that could potentially eliminate the fungi present on fruits[17]. Overall, using these two new substances can help eliminate *Monilinia fructicola* and similar species. Additionally, testing the combination treatment on actual stone fruits which are affected could be done in the future to see if the results produced are similar. Furthermore, increasing the concentration of each substance for the groups could also be done to see if the strength of the solution affects the results.

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## Appendix A

### Total Fungal Spores Before

| Control (x 10^8 spores/mL) | Whey (x 10^8 spores/mL) | Powdered Whole Milk (x 10^8 spores/mL) | Neem Oil (x 10^8 spores/mL) | Combination (x 10^8 spores/mL) |
|---|---|---|---|---|
| 2.72 | 3.98 | 3.76 | 3.91 | 4.09 |
| 1.15 | 3.85 | 2.76 | 3.07 | 3.76 |
| 8.71 | 4.05 | 4.32 | 3.29 | 5.12 |
| 1.21 | 6.12 | 5.12 | 4.67 | 3.87 |
| 1.38 | 5.89 | 4.67 | 3.88 | 3.74 |
| 1.48 | 3.89 | 4.78 | 3.9 | 5.23 |
| .97 | 5.56 | 5.89 | 4.22 | 4.04 |
| 2.16 | 5.73 | 5.32 | 5.11 | 4.25 |
| 2.35 | 5.68 | 5.27 | 2.25 | 4.00 |
| 3.49 | 4.99 | 5.76 | 2.79 | 5.80 |
| 4.33 | 2.45 | 2.45 | 5.45 | 3.61 |
| 4.84 | 5.98 | 2.13 | 5.09 | 5.57 |
| 6.68 | 1.11 | 3.47 | 2.32 | 4.01 |
| 1.95 | 5.09 | 4.16 | 5.00 | 4.99 |
| 3.23 | 5.13 | 4.19 | 4.91 | 3.77 |
| 6.01 | 2.92 | 4.28 | 3.27 | 3.22 |
| 4.00 | 3.10 | 4.24 | 3.01 | 4.29 |
| 2.08 | 3.27 | 4.45 | 4.12 | 3.45 |
| 1.90 | 5.34 | 3.21 | 3.98 | 5.12 |
| 1.09 | 2.78 | 3.27 | 3.84 | 4.03 |
| 1.35 | 5.11 | 3.45 | 4.35 | 5.67 |
| 1.68 | 4.82 | 4.68 | 3.27 | 3.79 |
| 1.01 | 4.97 | 2.14 | 4.11 | 6.02 |
| 1.24 | 4.31 | 5.23 | 3.00 | 4.10 |
| 1.51 | 1.78 | 6.54 | 3.47 | 3.91 |
| 2.59 | 4.46 | 6.12 | 3.32 | 2.87 |
| 2.39 | 4.37 | 3.83 | 5.17 | 4.02 |
| 1.47 | 3.82 | 6.84 | 4.12 | 3.70 |
| 2.00 | 7.12 | 3.55 | 5.93 | 4.04 |
| 1.81 | 4.08 | 4.98 | 5.01 | 5.10 |

## Total Fungal Spores After

| Control (x 10^8 spores/mL) | Whey (x 10^8 spores/mL) | Powdered Whole Milk (x 10^8 spores/mL) | Neem Oil (x 10^8 spores/mL) | Combination (x 10^8 spores/mL) |
|---|---|---|---|---|
| 3.12 | .07 | .14 | .11 | .10 |
| 1.60 | .06 | .08 | .08 | .04 |
| 9.23 | .09 | .12 | .07 | .07 |
| 1.52 | .50 | .18 | .15 | .06 |
| 1.62 | .35 | .22 | .08 | .04 |
| 1.71 | .07 | .57 | .09 | .18 |
| 1.22 | .48 | .22 | .11 | .08 |
| 2.40 | .36 | .43 | .30 | .11 |
| 2.42 | .35 | .30 | .15 | .09 |
| 4.07 | .29 | .28 | .22 | .05 |
| 4.37 | .15 | .41 | .48 | .04 |
| 4.82 | .47 | .17 | .33 | .04 |
| 6.71 | .49 | .04 | .55 | .06 |
| 2.10 | .36 | .08 | .32 | .01 |
| 3.29 | .38 | .16 | .47 | .04 |
| 6.05 | .09 | .25 | .08 | .01 |
| 4.01 | .07 | .38 | .02 | .11 |
| 2.16 | .29 | .13 | .39 | .19 |
| 2.21 | .30 | .15 | .29 | .16 |
| 1.12 | .03 | .05 | .22 | 0 |
| 1.42 | .30 | .08 | .33 | .08 |
| 1.83 | .22 | .18 | .03 | .01 |
| 1.01 | .24 | .20 | .12 | .01 |
| 1.29 | .29 | .20 | .02 | .10 |
| 1.73 | .15 | .39 | .30 | .01 |
| 2.75 | .12 | .04 | .23 | .02 |
| 2.46 | .12 | .46 | .29 | .09 |
| 1.52 | .14 | .39 | .12 | .07 |
| 2.06 | .42 | .14 | .27 | .09 |
| 1.95 | .10 | .13 | .04 | .02 |

## Tables/Figures

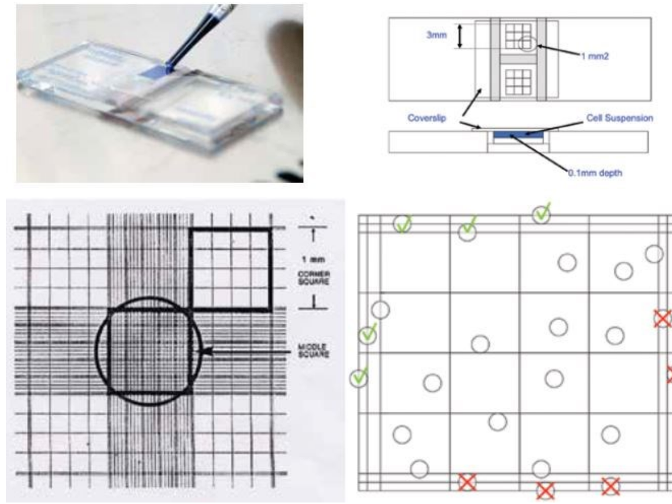


Figure #1 - Counting using hemocytometer[14.] Top left corner shows the hemocytometer, top right corner shows diagram on where the sections of the hemocytometer is, the bottom left corner shows the grid lines on the hemocytometer (enlarged view), and the bottom right corner shows how to count the fungi or bacteria using a hemocytometer.

## Experimental Design Diagram

**Title of the Experiment:** The effect of whey, *Azadirachta indica* (neem oil), and whole powdered whole milk on *Monilinia fructicola*.

**Hypothesis:** If the 2% concentration of the combined mixture of solutes (whey (v/v), *Azadirachta indica* (v/v), and powdered whole milk (w/v)) is added to the *Monilinia fructicola*, then the number of fungal spores will decrease the most because the mixture contains the benefits and characteristics of the 3 substances, eliminating the fungi most effectively.

**Independent Variable:** The types of the solutes (whey (v/v), *Azadirachta indica* (v/v), powdered whole milk(w/v), combination of all three (v/v)) in percentage.

| **Levels of Independent Variable** | 2% of solutes<br><br>Whey | 2% of solutes<br><br>*Azadirachta indica* | 2% of solutes<br><br>Powdered Whole Milk | 2% of solutes<br><br>Combination of all three (v/v) | Control (0% of solutes) |
|---|---|---|---|---|---|
| **Number of Repeated Trials** | 30 | 30 | 30 | 30 | 30 |

**Dependent Variable:** The amount of *Monilinia fructicola* present after the solutions have been put into effect (the number of fungal spores).

**Constants**: The type of fungus (*M. fructicola*), the type of fruit, the temperature and amount of days in the incubator, the amount of substances used for each item (20 mL of each to create original substance), the type of agar (potato dextrose agar), and a petri dish.

**Control:** *Monilinia fructicola* without any treatment.

**Table #1 - The mean before/after, and the mean difference among the treatment groups are shown.**

| Number of Spores Before and After (spores/mL) | Control (x 10^8 spores/mL) | Powdered Whole Milk (x 10^8 spores/mL) | *Azadirachta indica* oil (x 10^8 spores/mL) | Whey (x 10^8 spores/mL) | Combination (3 substances) (x 10^8 ) (spores/mL) |
|---|---|---|---|---|---|
| Mean Before | 2.626 | 4.362 | 3.9943 | 4.3917 | 4.306 |
| Mean After | 2.7923 | 0.219 | .20867 | .245 | .66 |
| Mean Difference | .1663 | -4.143 | -3.7857 | -4.1467 | -4.24 |

**Table #2 - This shows the observations, mean, standard deviation, and standard error mean for the control**

| | N | Mean (x 10^8 spores/mL) | StDev (x 10^8 spores/mL) | SE Mean (x 10^8 spores/mL) |
|---|---|---|---|---|
| Before | 30 | 2.626 | 1.8587 | .3394 |
| After | 30 | 2.7923 | 1.8772 | .3427 |

**Table #3 - This shows the observations, mean, standard deviation, and standard error mean for whey**

| | N | Mean (x 10^8 spores/mL) | StDev (x 10^8 spores/mL) | SE Mean (x 10^8 spores/mL) |
|---|---|---|---|---|
| Before | 30 | 4.3917 | 1.3602 | .2483 |
| After | 30 | .245 | .1489 | .0272 |

**Table #4 - This shows the observations, mean, standard deviation, and standard error mean for powdered whole milk.**

| | N | Mean x 10^8 (spores/mL) | StDev (x 10^8 spores/mL) | SE Mean (x 10^8 spores/mL) |
|---|---|---|---|---|
| Before | 30 | 4.362 | 1.2287 | .2243 |
| After | 30 | .219 | .1391 | .0254 |

**Table #5 - This shows the observations, mean, standard deviation, and standard error mean for neem oil**

| | N | Mean (x 10^8 spores/mL) | StDev (x 10^8 spores/mL) | SE Mean (x 10^8 spores/mL) |
|---|---|---|---|---|
| Before | 30 | 3.9943 | .9412 | .1718 |
| After | 30 | 0.2087 | .1467 | .0268 |

**Table #6 - This shows the observations, mean, standard deviation, and standard error mean for combination**

| | N | Mean (x 10^8 spores/mL) | StDev (x 10^8 spores/mL) | SE Mean (x 10^8 spores/mL) |
|---|---|---|---|---|
| Before | 30 | 4.306 | .8057 | 0.1471 |
| After | 30 | .066 | .0504 | 0.0092 |

**Figure #2**

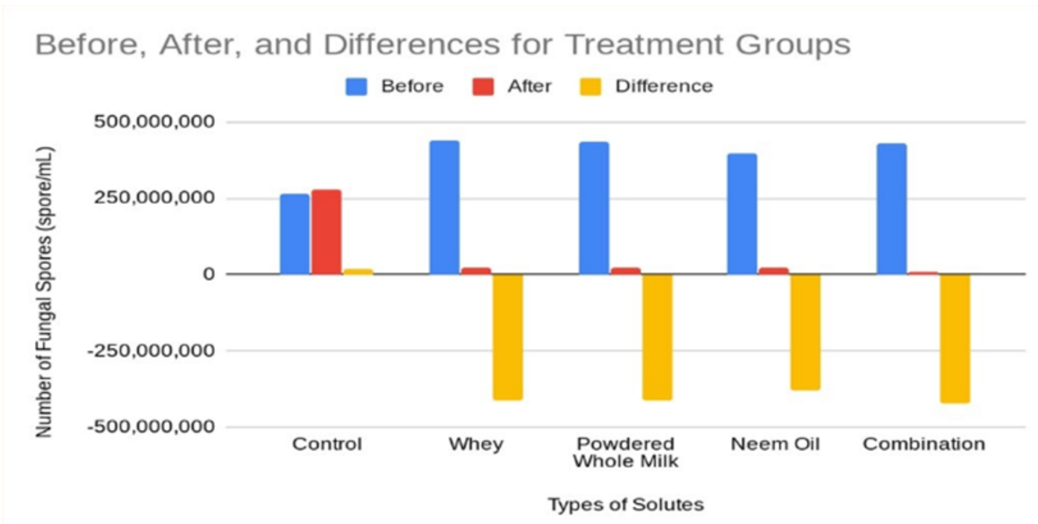


**Figure #3**