APPLICATION OF HYDROGEN PEROXIDE AND SODIUM CHLORIDE FOR THE CONTROL OF MOKO DISEASE *(Ralstonia solanacearum Yabuuchi et.al)* IN 'CAVENDISH' BANANA

[1]Jesryl B. Paulite, [1]Rosa C. Plaza and Nick Guilbert T. Pocon[2]
[1]Surigao del Sur State University-Tagbina Campus, 8308, Philippines
[2]Kimamon National High School, Kimamon, Sto Tomas, Davao del Norte, 8112, Philippines
Corresponding author: *Jesrylpaulite89@gmail.com*

Abstract
The study conducted to evaluate the effects of hydrogen peroxide (H_2O_2) and Sodium Chloride (NaCl) for the control of moko disease of 'Cavendish' banana; and To determine the effective treatments for the control of moko disease of 'Cavendish' banana. Results revealed that the percent (%) disease incidence of sterilized tap water and 100g sodium chloride attained the highest number of wilted plants in tissue cultured 'Cavendish' banana with the percentage of 98.25%, followed by 100 ml Hydrogen Peroxide alone with the percentage of 79.25% plants wilted while 1:1- 50 ml Hydrogen Peroxide and 50g NaCl) was comparable with Formaldehyde having a mean of 40.25%.

Keywords: hydrogen peroxide (H_2O_2); Moko disease; *Ralstonia solanacearum*; Sodium Chloride (NaCl)

1. INTRODUCTION

Banana is the most popular fruits in the world. Member of Genus Musa (part of the family *Musaceae)* were derived from the wild species (*Musa acuminate)* and (*Musa balbasiane).* It believes that there are almost 1,000 varieties of banana of the world subdivided into 50 groups. The commonly known banana is the 'Cavendish' (*Musa cavendishii*) variety, which is produce for export market (Morton, 2004).

Bananas are very important commodity for developing countries like Philippines. Together with rice, wheat, and maize and a Fundamental export commodity. 'Cavendish' banana are very sensitive commodity at the international level, not only on economical grounds but also on the environmental, social, political aspects (Mohan, 2006).

'Cavendish' banana is very susceptible to plant disease which results to remarkable loss in its marketable volume and quality. One of the very destructive diseases of 'Cavendish' banana is moko or bacterial wilt which is caused by *Ralstonia solanacearum* (Yabuuchi et.al) this disease is endemic to the Philippines, Central and South America. Control measures of this disease involves burning of the infected plant including the 5.3 meter radius around the infected mat to exclusive Quarantine for one month to avoid the spread of the disease when planted again after eradication. With this, the search for some alternatives to combat this disease to reduce the number of mats to be chopped is of its peak stages.

In the other hand, Hydrogen Peroxide (H_2O_2) is a clear liquid, slightly more viscous than water. In dilute solution, it appears colorless. Due to its oxidizing properties, H_2O_2 is often use as bleach or cleaning agent, wound dressing and mouthwash to kill bacteria contamination and infection. Organisms also naturally produce hydrogen peroxide as a by-product of oxidative metabolism. Consequently nearly all living things posses enzyme known as catalyses, peroxide which harmless and catalytically decompose low concentrations of hydrogen peroxide to water and oxygen. In addition, salt or Sodium chloride (NaCl) is applied to substance produce by the action of an acid with base, known as neutralization reaction. Salt are characterized by ionic bond, relatively high melting point, electrical conductivity (Schuber, Undated).

This study therefore was conducted to validate the claims on the hydrogen peroxide and sodium chloride against *Ralstonia solanacearum* Yabuuchi et. al causing moko disease of 'Cavendish' banana.

Objectives of the study

General objective:

To control moko disease of 'Cavendish' banana using the hydrogen peroxide (H2O2) and Sodium Chloride (NaCl).

Specific Objectives:

To evaluate the effects of hydrogen peroxide (H_2O_2) and Sodium Chloride (NaCl) for the control of moko disease of 'Cavendish' banana; and

To determine the effective treatments for the control of moko disease of 'Cavendish' banana.

2. METHODOLOGY

Location and Duration of the study

This research was conducted at newly established nursery at Capungagan National High school- KNHS Annex Field from November 25 to March 25, 2014.

Experimental Design and Treatments

A Complete Randomized Design (CRD) was used in the conduct of the study with five treatments and replicated five times.
The treatments were as follow:
T1-Sterile Tap Water
T2- 100ml Hydrogen Peroxide (H_2O_2)
T3- 100g Sodium Chloride (NaCl)
T4- 1:1 (50% H_2O_2 and 50% NaCl)
T5- Formaldehyde

***In Vitro* Test**
Specimen Collection

Specimen was collected from the area positively infected with moko disease caused by *Ralstonia solanacearum.* The corm part was then cut and placed in a double layered smooth cellophane for laboratory confirmation and detection.

Bacterial Ooze Test

Bananas infected with moko disease was collected from the field of Brgy. Capungagan, Kapalong, Davao del Norte. Infected plant part was cut and washed in running tap water and rinsed with sterilize distilled water. The infected portion hanged using paper unto the test tube and allow for ooze test for 3-5 minutes. This was done to check the presence of bacterial exudates.

Media Preparation

The soil medium was prepared using vermicast (1kilo/seedling pot) produced by the university bioreactor. The medium was properly screened, cleaned, before placing in the sack, with holes in the bottom for water drainage.

Isolation of *Ralstonia solanacearum*

Isolation was done at Polymerase Chain Reaction Laboratory of University of Southeastern Philippines-Tagum-Mabini Campus Science Laboratory. The bacterial suspension after ooze test was streaked in potato, dextrose, peptone, agar (PDPA). It is then incubated at 27 ^{0}C for 24 hours. As soon as the bacterial growth appeared, a single colony was transferred and streaked in PDPA and incubated.

Preparation of Bacterium suspension and Determination of Viable Cell Count

The 500 ml of *Ralstonia solanacearum* was prepared by collecting/dislodging their growth from three-day old pure culture using a sterile were loop. The bacterium suspension was then thoroughly mixed/shaken to distribute bacterial cells evenly. Bacterium suspension of *Ralstonia solanacearum* was standardized to $4x10^8$ cfu/ml using hemacytometer.

Flow Diagram

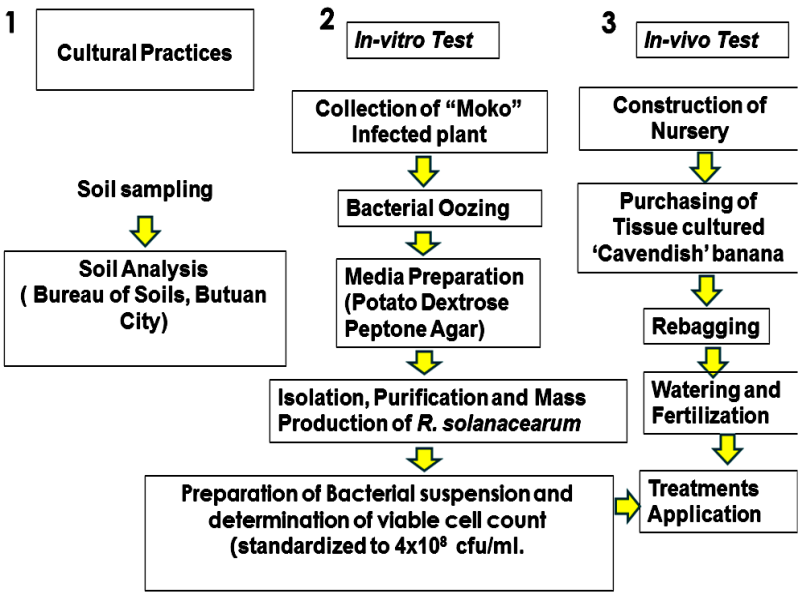

Figure 2. Actual application of hydrogen peroxide (left), Sodium chloride (center) and Formaldehyde (right).

Statistical Analysis

The Percent (%) incidence data were analyzed using the Analysis of Variance (ANOVA) of CRD and significant difference among treatments means were further analyzed using the Honestly Significant Difference (HSD) at 5% level of significance

3. DATA GATHERED

The data on Average plant height, number of functional leaf and percent (%) incidence on the Effects of Hydrogen Peroxide and Sodium Chloride for the Control of Moko Disease *(Ralstonia Solanacearum Yabuuchi et.al*) Of 'Cavendish' Banana is presented in table 1 and transformation of data using arc sine transformation before Analysis of Variance (ANOVA).

Table 1. Summary Of Data on the Effects of Hydrogen Peroxide and Sodium Chloride for the Control of Moko Disease *(Ralstonia Solanacearum Yabuuchi et.al)* Of 'Cavendish' Banana

TREATMENT	[1]Plant height (cm)[ns]	[1]No. of Functional Leaf [ns]	[2]Percent (%) Incidence*
T1 - Sterile Tap water alone	94.60	7.80	98.75[b]
T2 -100ml Hydrogen peroxide (H_2O_2)	95.20	8.00	79.25[ab]
T3- 100g Sodium Chloride (NaCl)	96.60	6.80	98.75[b]
T4- 1:1(50ml H_2O_2 and 50g NaCl)	93.00	7.60	40.25[a]
T5-Formaldehyde	85.40	7.40	40.25[a]
Grand Mean	92.96	7.52	71.45
CV (%)	8.10	10.30	54.58
HSD $_{(0.05)}$	-	-	50.06
HSD $_{(0.01)}$	-	-	77.27

Means having the same letter superscript are not significantly different at 5% level of significance using HSD.
[1]Data were untransformed and taken 90DAT
2Data were transformed using Arc Sine Transformation

Table 1 shows that the use of hydrogen peroxide and sodium chloride insignificantly affects the plant height and number of functional leaf of 'Cavendish' banana. Plant height and number of functional leaf are another parameters for determining growth. With this data it can be inferred that the control measure applied to 'cavendish' banana did not hinder with its growth, a desirable character for biocontrol agent. It dectates that the number of functional leaves were maintained or not significant since weekly 'cavendish' banana produces one leaf and stops when it reach to flowering stage. After flowering no leaves will arise from the plant. This is the time number when functional leaf is needed for photosynthetic activity reasons (Ploetz, 2000).

Moreover, the essential nutrients needed by plants in relatively small quantities are referred to as "micronutrients" or "trace elements". This include Fe, Zn, Cl, Mo, Mn, Cu, and B. Boron (B) is involved in the synthesis of ATP, translocation of sugar across cell membrane, and important in cell division. Iron (Fe) is associated with the synthesis of chloroplastic protein; Iron is also an enzyme activator and a component in Cytochrome. Manganese (Mn) is involved in enzyme activation important in carbohydrate metabolism, synthesis of riboflavin and carotene, synthesis of chlorophyll, and photolysis of water. Copper (Cu) is necessary in the formation of chlorophyll and an activator of several enzyme. Zinc (Zn) is involved in the synthesis of trypthopan, a precursor of indoleacetic acid (IAA), so it is involved in growth. Molybdenum (Mo) is required in N transformation such as nitrate-reduction within plant (Pava and Abellanosa, 2003).

Figure 3. The Actual measurement of plant height (upper left), the no. of number of functional leaf (upper right) and the percent (%) incidence (bottom).

Statistically revealed that, 1:1(50ml H_2O_2 and 50g NaCl) and formaldehyde having a comparable effects in terms of percent (%) incidence of 'Cavendish' banana having a mean of 40.25% and significantly different compared to sterile tap water, 100g NaCl and Hydrogen peroxide alone. The researcher also observed that when sodium chloride and hydrogen peroxide were used separately insignificant results appeared compared to the combination of both treatments.

Pareja (2012) states that, Moko disease is a bacterial wilt caused by *Ralstonia solanacearum* invading the vascular tissues of hosts. *Ralstonia solanacearum* is a species complex with exceptional diversity amongst strains from different hosts and geographical origins. The Hydrogen Peroxide is often use as an antiseptic and germicide for minor cuts and abrasions while sodium chloride decreases soil pH making it more acidic which is not favorable for the bacteria to growth (Bernstein, 1975 as cited by Pareja, 2012).

4. CONCLUSION AND RECOMMENDATION

The results of the study showed that the percent (%) disease incidence of sterilized tap water and 100g sodium chloride attained the highest number of wilted plants in tissue cultured 'Cavendish' banana with the percentage of 98.25%, followed by 100 ml Hydrogen Peroxide alone with the percentage of 79.25% plants wilted while 1:1- 50 ml Hydrogen Peroxide and 50g Sodium Chloride) was comparable with Formaldehyde having a mean of 40.25%, respectively.

Based on the results of the study, the author recommends similar field trial for further evaluation but in an increasing number of test plants per treatment application.

REFERENCES

Bernstein L (1975) Effects of salinity and Acidity on plant growth. *Annual Review of Phytopathology* 13, 295-312.

PAREJA, P.C, 2012- Different Levels of Vermitea for the control of Moko in Tissue Cultured 'Cavendish' Banana caused by (*Ralstonia solanacearum*). Unpublished Thesis,

PAVA H. AND ABELLANOSA A. 2003. Introduction To Crop Science, Third Edition. pp. 77-83.

Ploetz, R. C. 2000. Panama disease: A classic and destructive disease of na Obanana. Online. Plant Health Progress doi:10.1094/PHP-2000-1204-01-HM)