Final report -NSA 2020-English

Ministry of Agriculture & Forestry
Department of Agriculture
Plant Quarantine Division Ref:

Vientiane Capital: May 1, 2020

To: Director -Department of Agriculture
(Through administrative and organizational divisions)

Ref: Report of the successful implementation of the NSA WILT STOP Soil Conditioner to Control FUSARIUM WILT Topical Race 1 of banana plants in the field in Lao PDR 2019-2020.

According to a technical cooperation note between the Department of Agriculture, the Ministry of Agriculture and Forestry, the Lao PDR and the Soils North Pty Ltd, Australia, on the NSA WILT STOP trial program to control FUSARIUM WILT Tropical Race 1 of bananas plants in the field in Lao PDR 2019-2020.

Pursuant to the plan for implementing the 2019-2020 Organic Soil Conditioner Testing Program.

I. Introduction

Technical Cooperation between the Department of Agriculture, Ministry of Agriculture and Forestry, Laos PDR and Soils North Pty Ltd, Australia, on pilot performance of organic Soil Conditioner NSA WILT STOP to control the yellow disease (TR1) of bananas which signed the partnership agreement on 22 January 2019, with the aim to promote the implementation of pilot projects for Soil Conditioner efficiency use of NSA organic (100% Organic Soil Conditioner) in control of yellow disease (TR1) of banana plants in the field in Xaythany District, Vientiane Capital. Implementation of the project from agreement signing February 2019 to March 2020.

Pursuant to the Prime Minister's Directive on Increasing Prevention, Control and Preparedness Measures for (COVID-19) No. 06 / ນຍ dated 29/03/2020, the Technical Committee has postponed its plan for the data collection for last three months of the project.

II. The experiment purpose

To test the efficacy of NSA from Australia in control of TR1 strains in banana plants.

To study the proper and effective application rate of NSA.

III. Trial budget

Soils North Pty Ltd, Australia, has funded the project in detail as follows:

Total Budget: $A156,700 924,530,000 KIP.
These include:
- The budget obtained through the Department of Agriculture for the technical work of 126,349,000 KIP.
- The budget for the equipment for implementation of the project for the Project Supervisor, Staffs of Xaythany District and Vang Vieng District (2 motorcycles, 3 laptops, 2 printers) totaling 40,178,000 Kip.
- Site costs, plant supplies, NSA supply freight and customs, site labour and materials, including pumps, irrigations systems, plant hire, fencing, signage, fertiliser, soil amendments, pest treatment, staff and all associated costs 758,003,000 KIP.

IV. Implementation results

A. In terms of document preparation and data collection
- Complete the project management committee from the Department of Agriculture, the District Agriculture Office to coordinate and implement the project;
- Complete documentation and licensing to import of organic Soil Conditioner products for trial in accordance with the laws and regulations of Lao PDR;
- Successfully completed meeting between the technical staffs and company to develop project plan for pilot project in the field at Xaythany District, Vientiane Capital (Research proposal for the NSA Soil Conditioner application to control the yellow disease of bananas in details from soil preparation to the project completion).
- Complete field preparation and banana plantation ceremony, providing assistance to district staff in Xaythany district, Vientiane Capital. Planting was completed at the end of June 2019 and data collection began at the end of July 2019;
- Completed the field report of banana growth, assessing yellow disease in Thong Mang village, Xaythany district, six times, once a month (7, 8, 9, 10, 11, 12, 2019).
- Completed the meeting to report the implementation progress of the NSA organic Soil Conditioner trial for controlling the Epidemiology of banana disease in the field in Lao PDR held on 22/1/2020 at the Department of Agriculture;
- Completed the Banana Growth Field Report, assessing the yellow disease at Xaythany District for the month of 1, 2, 3, 2020.

B. Study and experimental results

Ban Thong Mang Village, Xaythany District, Vientiane Capital.

Field trial results of the NSA Soil Conditioner on the control of yellow disease of banana growth in Xaythany District, Vientiane Capital using different rates of NSA Soil Conditioner at the soils preparation stage (T1 - 3 T / ha, (T2 - 2 T / ha, (T3 - 1ton/ha and T4 - No Soil Conditioner. For both data collection, the two areas were performed once per month by the first observation and the number of early signs of TR1 and were measured, recorded, the height and circumference of the stems by randomly taking 10 trees / row.

i) The results of the NSA Soil Conditioner study on banana diseases, Pak Song variety of banana over a period of 9 months (June 2019 to March 2020) are summarized as follows:

Efficiency test of NSA organic Soil Conditioner to control the banana disease Foc race1 with Pak Song banana species (sugar banana) in Ban Thong Mang, Xaythany district, Vientiane, Capital with 4 trials and 3 replicates, after 9 months of data collection: each test

T1 with 3tonnes/ha,
T2 with 2 tonnes/ha,
T3 with 1ton/ha, and
T4 as a control, no NSA.

The plants were strong and showed no signs of yellow disease and no difference was found in the experimental conditions.
Instances of other banana diseases were present including black spot disease and yellow sigatoka leaf disease.
Of note all banana in T4 died. Seedlings had insufficient root growth when planted.

ii) Effectiveness of NSA Soil Conditioner on the growth of sugar banana in Ban Thong Mang village, Xaythany district.

As a result of data collecting the height and circumference of the banana trees, T1, which applied 3 tonnes / ha, improved significantly over the other trials in 1 to 9-month-old bananas, followed by T2, T3 and T4, respectively (Table 2).

The data found that the 3 tonnes of NSA/ha is highly effective for stem growth, and it can be concluded that the Soil Conditioner is highly effective for high yield.

V. Evaluate and summarize the implementation of the project

<u>Advantages:</u>
- Get regular attention from the Steering Committee for improvement;
- Get cooperation and support from provincial, district and technical levels;
- Get well supported by the company in the implementation;

- Exchanged and learned the technical aspects of the technique from the company and the technical staffs from the Central, provinces and districts levels.

Constraints:

- Changes to the data collection period are too short.
- Problems of probation: dry weather, and farmer did not adequately water the banana.
- NSA spread by hand and not even spreading.
- Banana seedlings supplied in 2 batches. Second batch had poor root growth and most did not survive. Batch 2 was planted in T4 and all banana plants died.
- Quarantine controls not practiced by the famer.

Lessons learned:

- Technical knowledge between technical staffs from the company and the agriculture technical staffs from the Central, provinces and districts to implement the project.
- Field trial planning should be tailored to the budget that the company has in order to provide complete and accurate information;
- The first and short-term trial is not yet clear but preliminary data, presented in the laboratory proposes to continue the trial based on this baseline and proposes a more complete plan;
- So far no disease TR1 has been found in the sugar banana Pak Song 50 species, proposing to continue comparing this species with others different types of banana with other strains to determine NSA effectiveness with other types of bananas.

Report of the NSA WILT STOP Organic Soil Conditioner Test in Controlling Banana Epidemic Disease.

1. Excerpt

A total of 45 banana samples were analyzed, including samples from banana farm, Thong Mang Village, Xaythany District, Vientiane Capital, 20 samples were purified using pure dilution pate and tissue culture, and the Molecular cell analysis was performed. sp. cubense race 1. Efficiency test of NSA organic Soil Conditioner in control of sugar banana Pak song 50 species ThongMang village, Xaythany district, Vientiane Capital, with 4 trials and 3 replicates, after 9 months of data collection: each T4 -control (No NSA) T1 with 3 tonnes/ha, T2 with 2 tonnes/ha and T3 with 1 tonnes/ha, the plants were strong and showed no signs of infection and each trial was no different.

2. Purpose of the study

- To study the causes of banana disease in sugar banana plantation in Thong Mang Village, Xaythany district, Vientiane Capital
- To study the effectiveness of NSA WILT STOP organic Soil Conditioner in the prevention of Foc race1.

3. Materials in the field and laboratory

► **Equipment and chemicals in the field**
- pick, knives, plastic bags, scissors, Silica gel, ice cube, cameras, 70 liquors and more.

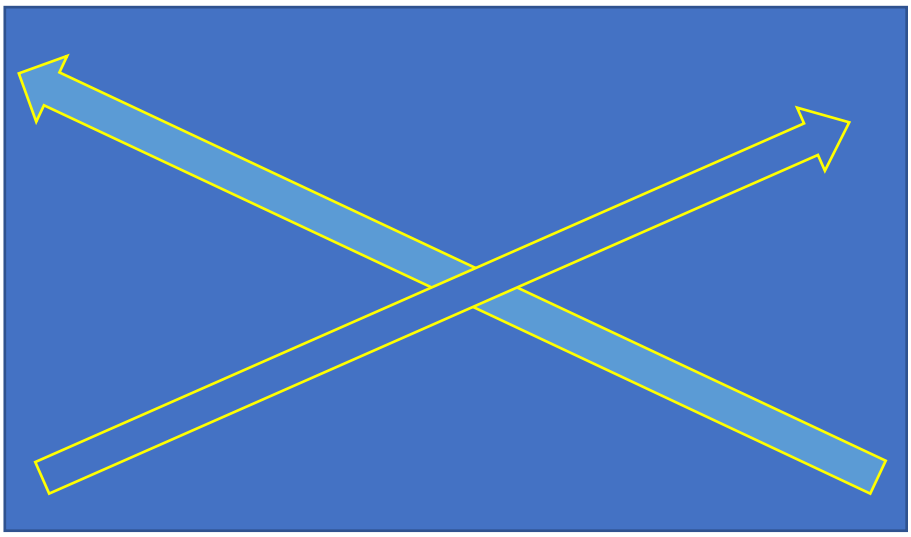
► **Laboratory equipment and chemicals**
- Antibiotics, DNA extraction kits, microscopes, PCR machines, Genetics, Primers and more.

4. Application

► **Methods for surveying and sampling of banana yellow disease**

- The survey is a cross-sectional walking examination to find the symptoms of infected plants: yellow leaf from the lower leaf to the shoot, when cutting from the trunk, it can be found the brown spots in the trunk and in the feeding tube.
- Sampling should be between 3 and 4 samples in an infected area.
- Slice the inner portion of the banana in color length 4 to 5 by 5 to 8. Place on a vacuum or sanitizer paper, set on Silica gel, leave to dry and turn Silica gel when seen in pink.
- If the specimen is well dried, put it in an envelope and record the date of collection, collector's name, sample code, background, varieties and more.
- Soil samples were collected to investigate the pathogens.
- After survey and sampling of each day and the samples must be separated.

Diagrams for the exploration and banana disease sampling



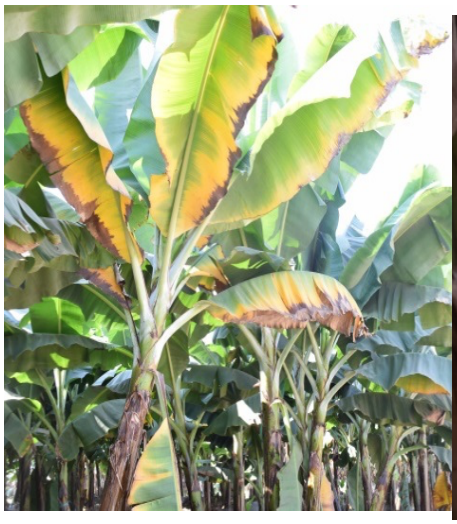
Symptoms of Banana yellow Diseases



► **Isolation of pure germs from soil**

► **Isolation of pure germs from plant tissue**

- Cut the parts of the wound area to about one half of the infected area, generally cutting each piece to a size of 4x4 millimeters.
- Disinfected at the surface of the plant, most commonly using (sodium hypochlorite solution 3%).
- Put the sliced plant parts in the solvent for 2-5 minutes, rinse with sterile distilled water for 2-3 times, and then dry with filter paper that already baked.
- Then put the disinfected plant parts on the feed agar can be put in several different ways, like 4 points, with each plant apart to allow the germs to grow from each part of the crop in water agar.



- Absorb 1 ml of water but put in rose bangal, leave it in room for 3-7 days.
- Observe the white spores and cut out the solitary filaments in the PDA dish, using the microscope and counting the number of fungus strains of Fusarium oxysporum f. sp.

DNA extraction and analysis using the (Pure Dire Gernomic DNA Isolation kit)

Put the pure Fusarium sp into 1.5 ml, add 50 µL of CR buffer, add 300 µL of CC buffer and mix, stirring at 60oC for 10 minutes during incubation should be stirred every 3 minutes and left in a regular room for 1 night. Add 400 µL of mixed buffer CB and leave at 4oC for 1 hour, 12,000 x g centrifuge for 1 minute. Extract with column CC and centrifuge 14,000 x g for 30 seconds, then drain off water. Add 400 µL of buffer W1 and then centrifuge at 14,000 x g for 30 seconds. Add 600 µL of buffer W2 (Ethanol added) and Centrifuge 14,000 x g for 30 seconds and then drain 14,000 x g centrifuge for 2 minutes then drain buffer W2 again. Move dried column to 1.5 ml micro centrifuge tube. Add 150 µL of BE to the middle of the column matrix and centrifuge 14,000 x g for 2 minutes to obtain pure DNA.

Components of analysis in PCR machines

PCR Reagents	1x (µL)	PCR Condition	
		Temp. (°C)	Time
Green Hot Start Master Mixed	10		
F primer TR4 (10uM)	1	95	5 mins
R primer TR4 (10uM)	1	95	1 min
F primer EF1 (10uM)	1	60	1 min
R primer EF2 (10uM)	1	72	3 min
Water	5	72	10 mins
Total	20	30 cycles	

► **NSA Organic Soil Conditioner Study to Control Banana Diseases in Thong Mang Village, Xaythany District, Vientiane Capital**

♦ **Study Plans**

The variety used in the NSA test for yellow disease control of Foc race1 was Sugar Banana Pak Song 50 species which plan in T-test designs with 4 trials and 3 replicates and 1 duplicate has 10 test units: T1 was 3 tonnes/1 ha, T2 was 2 tonnes /1 ha, T3 was 1 tonne/ha and T4 was control no NSA.

♦ **Evaluation of Banana Epidemic Outbreak**

Data collection starts from the month of June 2019 to March 2020 The data collection is surveyed every one by rating the symptoms of leaves yellowing 0-4: 0 is banana still has no symptoms, 1 is 1- 2 started yellow leaves, 2 has 1-2 yellow leaves and broken stems, 3 has 3 or more yellow leaves and broken stems at the height of 20cm and 4 is the plants died.

5. Results of the study

♦ **Results of Morphology and DNA Research**

Morphology and DNA analysis results using the TR4 specific primer after collecting soil samples and samples from banana trees and found: samples taken from Thong Mang village, Xaythany District, Vientiane Capital, 15 samples showed an average of 1x101 low soil contamination and 0 banana plants samples found Foc.

♦ **NSA Soil Conditioner application study to control the banana yellow disease at Ban Thong Mang Village, Vientiane capital**

Table 1. Infection Percent of Foc Race1 in banana trees

Test method	Severity percentage of Foc Race 1 in banana plants
T1	0
T2	0
T3	0
T4(control)	All control die

Through monitoring and evaluation of the Foc race1 virus infection with the Pak Song variety 50, and data collection from the month of June 2019 to March 2020, the NSA Soil Conditioner test to control the disease of banana Foc race1 with the Pak Song variety 50 at Thong Mang village, Vientiane capital, there were four trials and three replicates: T1 was 3 tonnes / 1 ha, T2 with 2 tonnes / ha and T3 with 1 tonne / ha and T4 control (no NSA-Soil Conditioner), noted that banana plants are strong and have no symptoms of the disease mostly black spot disease and yellow sigatoka leaf disease. Note comment T4 seedlings died.

Test plants at Ban Thong Mang, Saythany District, Vientiane Capital

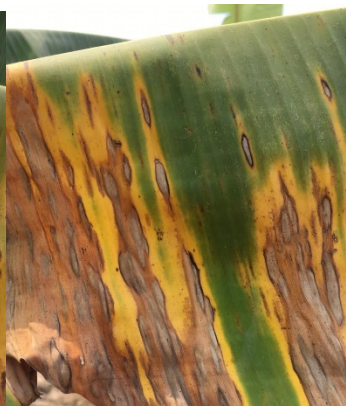


Table 2 Growth of banana in Ban Thong Mang village, Xaythany district, Vientiane Capital (using sugar banana Pak Song variety 50)

Test Method	1 mth		2 mth		3 mth		4 mth		5 mth		6 mth		7 mth		8 mth		9 mth	
	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm	H cm	C cm
T1	36.4	14.1	98.4	26.6	140	40.1	163.8	51.6	176.3	55.8	174.8	51.9	178.6	53.8	182	52.5	187	50
T2	31.6	14.1	72.5	21.1	137.3	38.8	158.6	50.6	164.5	53	173	52.4	168.2	48.2	175.9	52	177	48.7
T3	29.1	12.7	94.5	26.6	136.9	38.4	167	50.3	170	56.9	172.5	53.4	162.4	54.6	174	55	176	50
T4 (Control)	27.4	11.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

H - Tree height

C - Tree circumference