# Development of Pre - Detection Technique for Bacterial Blight (*Xanthomonas axonopodis pv.dieffenbachiae*) Disease in Anthurium to Produce Healthy Planting Materials.

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## ABSTRACT

**Anthurium cut-flowers and potted plants have earned a growing marketing demand both in the local and global markets. Bacterial Blight, caused by *Xanthomonas axonopodis pv. dieffenbachiae* has been of major economic concern among the growers world wide as the disease could cause heavy losses on Anthuriums and other members of the family Araceae. The disease has the potential of spreading latently exhibiting no symptoms through a range of hosts and this fact makes it a pre-requisite to detect the presence of pathogen early. No reliable control method so far has been developed and therefore, maintenance of strict crop sanitary measures is of great importance. 100 samples representing 30 nurseries were selected from three of the major producing districts, Gampaha, Kurunegala and Puttlam districts. The pathogen was isolated from the samples using 5% KOH test and Yeast Dextrose Calcium carbonate medium. Isolated pathogen was inoculated to healthy Dieffenbachia cane and leaf slices and after 1-2 days, symptoms first developed in cane cuttings while leaves took 3 days for the development of symptoms. Young, tender leaf and cane slices were observed to be highly susceptible to disease. The mature cane slices and leaf cuttings were found to be resistant to the pathogen, most probably due to the non-specific mechanical impermeability of the tissues. Tender leaf and cane tissues, with their ability to get infected and develop visual symptoms relatively fast, worked best with the technique as they proved to shorten the time taken for the detection. The validity and the precision of the pathogen identification test and therefore, the method, were assessed with a Indirect -ELISA pathogen-specific detection step, prior to inoculation of healthy tissues. Pathogen was detected to present in 15 samples from Kurunegala and Gampaha districts. The method confirmed its precision, reliability, cost-effectiveness and application under normal laboratory conditions.**

**KEYWORDS:** Anthurium, Araceae, Bacterial Blight, Pre- Detection Technique, *Xanthomonas axonopodis pv. dieffenbachiae*

## INTRODUCTION

Anthurium is very much popular among Sri Lankan growers as a cut flower. During recent years its importance has further increased as a potted plant.

Anthuriums are being grown commercially for exports as well as for the local market. Annual production is around 3 million flowers, the majority of which are sold at the local market. Although the current exports of Anthurium are not very much significant, it has a great potential for the future as a key foreign exchange generator. Total land area at present under cultivation is around 10ha, and the industry keeps expanding at the village level.

During recent years, exports of floricultural products have shown a considerable increase.

The main export markets of Sri Lankan Anthuriums include Europe (72%), Far East and Middle East (28%) (Dhanasekara, 1998). Sri Lanka's varied and stable tropical climatic conditions and the geographic terrains from sea level up to 2,200m of humid mounts have created magnificent macro and micro environments to house high quality Anthuriums. The country also has a favorable location to serve different markets in the world. Availability of land and high literacy rate of the average person would be an added benefit for those who wish to invest in the industry. In addition, the tax benefits and BOI incentives granted by the government would help bring in more investors to the country and facilitate further development of the existing industry. In order to establish a sustainable development, it is of very much importance to identify the current and future constraints of the industry. Biological impacts are of prime significance in this regard as pests and diseases can possibly terminate once flourished industries leaving heavy economic losses behind.

Bacterial blight, a disease of strict global concern, has been reported from Sri Lanka and if adequate preventive measures are not employed, it has the potential of resulting in a permanent collapse in the industry. It is one of the most serious diseases in the Anthurium cut-flower industry and caused by the pathogen *Xanthomonas axonopodis pv. dieffenbachiae* (previously, *X.campestris pv .dieffenbachiae*). This pathogen has many hosts in the family Araceae; *Anthurium, Dieffenbachia, Aglaonema, Philodendron, Colocasia, Caladium, Epipremnum, Xanthosoma* etc.(Cooksey,1985; Chase,

1987; Chase *et al.*,1988) and most of the Anthurium cultivars are susceptible to the disease, making it very difficult to manage once introduced to a production area.

The impact of the disease varies from extremely severe to a mere nuisance. Because of the systemic nature of the disease, it has proved to be difficult to control, especially if standard preventive and sanitary measures are not implemented early.

The disease was first described on *Dieffenbachia maculata* by McCulloch & Pirone (1939). Infection and increase of the disease take place especially under warm (>25°C) and humid conditions. The disease on Anthurium, however, is so devastating that a sudden outbreak in a new growing area may result in 50-100% loss of plants.

Natural dispersal of the bacterium is only on a very local scale. The most likely pathway for international movement is through planting or breeding material of ornamental Araceae, which may be latently infected. The broader range of ornamental hosts increases the risk of potential outbreaks in pathogen-free regions. This also applies to material in tissue culture; Norman & Alvarez (1994b) found that the bacterium could survive on apparently healthy material of *A.andreanum* in tissue culture for over a year.

The disease is of major concern among the growers due to heavy losses it can bring about. Symptoms of blight can easily be confused with those of other diseases, making a laboratory confirmation obligatory.

Phytosanitory risk behind the *X.axonopodis pv.dieffenbachiae (Xad)* is so concerned that it is considered a top level quarantine organism world over. The presence of the bacterium can be checked by isolation on selective medium, serological tests using monoclonal antibodies and a host test (Alvarez *et al.*, 1988; Norman & Alvarez, 1989; Lipp *et al.*, 1992; Norman & Alvarez, 1994a).

So far, no effective technique or chemical has been developed for the rapid and precise detection of the pathogen.

This study therefore, specifically targets the development of a rapid pre-detection technique for pathogen which will lead to identify infected (latent) planting material. As current serological tests based on antibodies are expensive and time consuming, this would be beneficial for the average grower.

## MATERIALS AND METHODS

**1. *Preliminary Survey and Sample Collection.***

A questionnaire was constructed to gather the information on the presence of the disease and it was introduced to commercial growers in three districts, namely Puttlam, Gampaha and Kurunegala. Thus, 30 nurseries were used to source necessary information on the occurrence of disease and later suspicious samples were collected randomly from each nursery. Samples from different Anthurium varieties were collected and finally, all together, there were 100 samples. Samples were separately stored in polythene bags and kept sealed at 4 C° until use.

**2. *Culturing.***

Each sample was thoroughly cleaned and washed with running water followed by a surface sterilization with 1% bleach solution. Next, they were again washed with sterilized distilled water prior to culturing on Potato Dextrose Agar (PDA) media under Laminar Flow. Sterilized Petri dishes were used for culturing and the cultures were incubated at 37 C°. During this step, normal surface sterilization procedures were followed with 70% Ethanol and autoclaved glassware and tools were used in order to minimize potential contamination threats.

**3. *Identification Tests.***

**3.1. KOH Test.**

Grown bacteria were streaked on 5% KOH solution on a slide (Lelliott and Stead, 1987).

**3.2. Yeast Dextrose Calcium Carbonate (YDC) Medium Test.**

Samples with positive results for KOH test were cultured on YDC media (Jeans, Pittsley, and Senti, 1961). Grown bacteria were diluted to obtain single colonies. Serial dilution technique was used. Cultures were finally incubated at 37 C°.

**3.3. Culturing in Nutrient Broth (NB) medium.**

Bacterial colonies which showed YDC positive results were separately cultured on NB media and finally incubated at 37C°.

**3.4. Indirect ELISA (Enzyme-Linked Immuno Sorbant Assay) Test.**

Confirmation test for the pathogen was conducted using the Bacterial reagent set- Indirect ELISA, alkaline phosphatase label by **agdia®** and the manufacturer-recommended standard protocol of the kit was followed.

Thirty two bacterial samples were tested with positive and negative controls along with buffer sample.

***4. Development of pre- detection technique for Bacterial blight using Dieffenbachia plants.***

Healthy Dieffenbachia cane and leaf tissues were placed in petri plates containing PDA with Chloramphenicol as an antibacterial agent. Each Petri dish had either for of the cane slices or four of the leaf cuttings. Leaf slices were damaged before placing in Petri plates. Both cane and leaf slices were inoculated separately with the most virulent two *Xad* samples, confirmed by indirect ELISA test. For both cane and leaf parts, 10 replicates were applied separately with controls. Petri plates were sealed and kept at 37 C° for incubation and observed for the development of disease. Healthy cane and leaf slices inoculated with sterilized distilled water were used as the control.

## RESULTS AND DISCUSSION

### *3. Identification Tests.*

### 3.1. KOH Test.

Gram negative bacteria form slimy and sticky threads in 5% KOH solution (Lelliott and Stead, 1987). As the pathogen under study is also a gram negative species, this test was first conducted to screen only the Gram negative bacteria. Fifty one cultured bacterial samples were found to give positive results at this step. The rest of the samples were discarded.

### 3.2. Yeast Dextrose Calcium Carbonate (YDC) Medium Test.

Out of all Gram negative bacterial samples cultured on YDC, thirty two developed yellow colored and shiny colonies. At low concentrations of bacteria, developed colonies were separate while at higher concentrations, they were not. *Xanthomonas spp.* form yellow colored and glossy colonies on YDC medium (Jeans,.Pittsley, and Senti, 1961). Thus, this indicated that the bacteria in thirty two samples could be *Xanthomonas spp.*

### 3.3. Culturing in Nutrient Broth (NB) medium.

Grown bacterial samples reduced the transparency of the medium assuring a proper growth.

### 3.4. Indirect ELISA (Enzyme Linked Immuno Sorbant Assay) Test.

From 32 YDC positive samples, 15 samples were observed to form the specific yellow color similar to the positive control in ELISA test. This test specifically detects the pathogen and therefore, this step confirmed specifically and accurately that these 15 samples contained the pathogen, *Xanthomonas axonopodis pv. dieffenbachiae.*

**Table 1 - Results of tests. (KOH test, YDC media test and ELISA test:**

| Districts | No. of samples | KOH positive | Yellow colour on YDC media | ElISA |
|---|---|---|---|---|
| Kurunegala | 52 | 29 | 18 | 14 |
| Puttlam | 30 | 10 | 6 | - |
| Gampaha | 18 | 12 | 8 | 1 |
| Total | 100 | 51 | 32 | 15 |

Dieffenbachia cane slices are more damaged compared to its leaf slices. Inoculated cane slices developed symptoms more rapidly in just 1-2 days. Leaf parts took 3 days for the symptom development. Some factors, such as nutrients in phloem sap and minerals in the xylem could create a pathogen-friendly environment in the cane, so the pathogen multiplies fast in tissue developing visible symptoms more rapidly. In addition to these, there may be some unknown factors which set a rapid acceleration in the pathogen population. In Dieffenbachia, the bacterium seems to be having some tissue specificity according to above mentioned observations.

Leaves may have barriers and chemical compounds that retard or delay the bacterial growth. This suggests that the Dieffenbachia cane slices are better than the leaf cuttings as they produce symptoms quickly.

Tender leaves and cane slices have shown to be highly susceptible to disease. Such tissues contain rapidly dividing parenchyma cells which are rich in food reserves. Such tender tissues are privileged by a continuous food supply and they have thin-walled cells. These cells therefore, are highly penetrable and provide the pathogen with an ideal environment to thrive. Incorporation of tender plant tissues in to this method can be advantageous as they would develop the symptoms fast and effectively: further cutting down the time requirement.

By adding Chloramphenicol to PDA, it is possible to avoid the bacterial growth in the medium and it facilitates proper growth of *Xad* on Dieffenbachia cane and leaf slices. *Xanthomonas spp.* form yellow-colored and glossy colonies on the YDC medium. Medium does not specifically detect the presence of the pathogen as there may be other bacteria which also develop colonies of the same color and texture. As samples come from various geoclimatological regions of the island and the globe, they may also contain many other known and unknown bacterial and other microbe forms, making the testing procedure more complex and possibly resulting in erroneous conclusion. A greater consideration should be focused on this fact as this medium test only help draw inferences that the *Xanthomonas spp.* may be present in the specimens under testing.

***Development of a pre-detection technique for Bacterial Blight using Dieffenbachia plant***

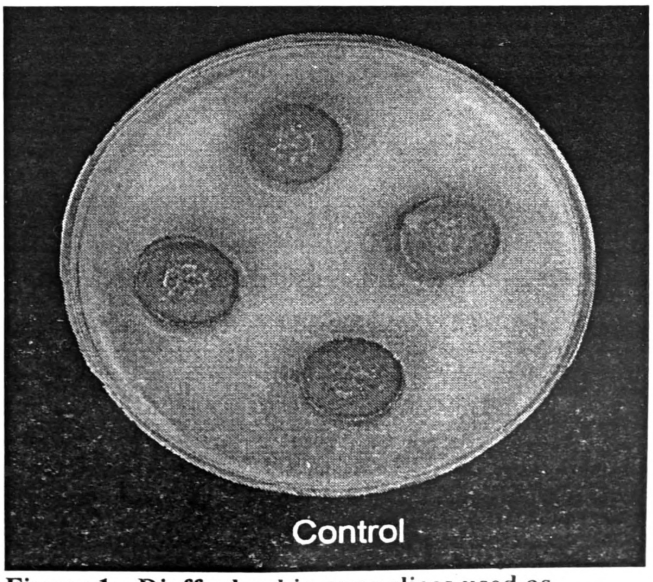


**Figure 1 - Dieffenbachia cane slices used as control:**

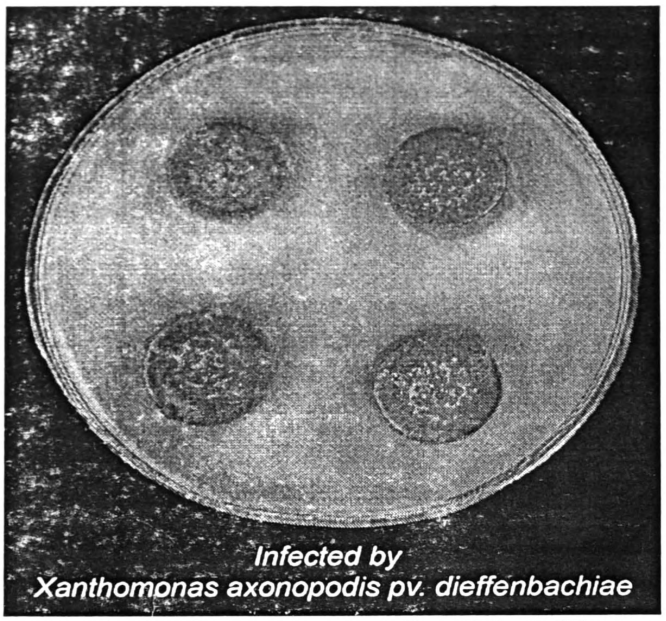


**Figure 2 - Dieffenbachia cane slices infected by *Xad:***

ELISA test was conducted during the work only as a confirmation test for the pathogen in order to increase the validity and the precision of the developed technique. ELISA test is expensive and for one-sample check, it will cost around 400 SLR (4 US$). The developed technique comprises of all other screening tests except the ELISA as the idea was to develop a cost-effective and reliable method that demands practical applications under average laboratory conditions.

Disease was found in 6 nurseries in Kurunegala and Gampaha districts. This suggests that the disease occurs in the nurseries in Kurunegala and Gampaha districts. Therefore, these nurseries should not distribute planting materials and growers should be properly educated in order to prevent the island-wide circulation of the pathogen. Imported ornamental planting material should be thoroughly inspected prior to the release to growers. All Aroids should be given a higher priority during quarantine process. From all the Anthurium varieties that are grown in nurseries, variety Midori showed tolerance to Bacterial Blight disease. All other varieties are highly susceptible to the disease.

Availability of large number of hosts, sanitation, relative humidity and application of higher rates of fertilizer will greatly affect on spreading of pathogen (Chase and Poole, 1986).

This method was developed using only one susceptible Aroid, Dieffenbachia, due to time restrictions. But more suitable species may still exist and having identified them, the method could be further streamlined to generate more promising results. It is always better to select the highly susceptible species.

Also this technique is cost-effective and demands average laboratory facilities. Although this method requires about one week time period, one can obtain satisfactory results. It requires less advanced technical knowledge. Possibilities of causing harmful effects are very less compared to serological methods. Prior to applying this method, it requires KOH test and growing on YDC media to identify the bacteria in species level.

Anthurium cuttings can not be used with this method as they contain phenolic compounds which oxidize once cut and mask the bacterial symptoms developed. And also, Anthurium tissues are much harder so that it takes more time to develop the disease symptoms. Dieffenbachia was found to be free of such problems and readily available. The method was, therefore, based upon this Aroid. There may be other host plants which yield more advantages and could be used to further rectify the procedure, so the effectiveness and accuracy would increase.

## CONCLUSIONS

The disease was identified to occur mainly in Kurunegala and Gampaha districts.

Only one Anthurium variety called Midori, showed the tolerance to this disease. All other varieties showed higher susceptibility to Bacterial blight.

Dieffenbachia tender cane slices were successfully used for pre-detection of Bacterial blight disease in Anthurium. Cost effectiveness and practicability under average laboratory conditions are the major important factors in this developed technique.

This can be further improved, by applying this method to different host plants of this pathogen.

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