

Appendix 6. Anova for color of polyuron treated with 50, 60, and 80% sweet potato flour.

Attributes Samples	A	B	C	D
Color	5.7	5.2	4.7	7.1
Texture	5.7	5.7	5.2	7.2
Flavor	6	5.5	4.8	7.3
Aroma	6.1	6.3	6.2	6.7
General Acceptability	0.57	5.6	5.1	7.3

LOCAL PAPERS AS SUBSTITUTE FOR NITROCELLULOSE MEMBRANE IN THE DETECTION OF BACTERIAL WILT LATENT INFECTION OF POTATO

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ABSTRACT

Eight locally produced papers were evaluated as possible substitute for expensive and imported nitrocellulose membrane. NCM - ELISA, a method of bacterial wilt testing developed by CIP, Lima, Peru was used as guide in the conduct of the study.

Among the materials tested, three gave promising results. These were Cactus Xerox paper, Ordinary Xerox Paper and Vernal Mimeographing paper (Substance 20). The purple coloration of the papers which corresponded to positive results were comparable or the same as that in Nitrocellulose membrane. It was found out that the antisera for the test could be recycled or utilized once.

Based on the results of the test, it was observed that 38.63% of the tubers of plants adjacent to bacterial wilt - infected hills were latently infected with the disease.

INTRODUCTION

Bacterial wilt disease incidence in Benguet and in Mindanao causes crop damage as high as 70 - 100%. Plants with latent infection do not show any visual symptoms of the disease. This situation poses a serious threat in the seed potato production because it is hard to pinpoint which among apparently - healthy - looking plants are infected.

The detection of latent infection can now be done by the use of expensive and imported materials like nitrocellulose membrane. Hence, alternative materials have been taken into considerations and the possibility

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of recycling or re - using the anti - sera was conceived to reduce the cost of testing.

OBJECTIVES

1. To find a cheaper material that could be used in lieu of expensive and imported nitrocellulose membrane as a support in the serological reaction of bacterial wilt test.
2. To determine what kind and substance of paper is best suited for the test.
3. To know how many times can the antisera be recycled.
4. To determine the percentage latent infection of tubers of plants adjacent to bacterial wilt infected hills.

METHODOLOGY

A. The Use of Local Papers :

Eight locally produced papers were used in this experiment, four of which were mimeographing papers; two Xerox papers. These were collected from newly opened "ream". Gloves were used as to avoid finger prints which may cause unspecific reactions. The papers were cut in the same size as the nitrocellulose membrane (NCM). The method of testing (NCM - ELISA) developed by CIP, Lima, Peru was used in the conduct of this experiment (Figure 1).

The tubers used were collected from the neighboring hills of bacterial wilt infected plants. These were washed thoroughly to remove all the soil clinging on the surface. Each tuber served as a sample. Using a scalpel, which was flamed sterilized every after use, the hill end and the rose end

were removed and placed in clean plastic bags. These were then macerated and added with extraction buffer at 1:1 ration. The crude sap with buffer was allowed to stand for 30 minutes, centrifuged at 1000 rpm for 2 minutes to allow starch and other sediments to settle.

An alteration in blotting technique was made, that is, blotting the sample on the dry surface of the papers since the sap samples tended to coalesce when the paper was soaked in Tris - buffer solution. Regarding the nitrocellulose membrane, the usual wetting with buffer before blotting was done.

Ten microliter (0.01 ml) of the crude sap was blotted in each of the 8 mm squares of the papers. The blotted papers were allowed to dry overnight. After drying, blocking with non - fat milk to fill spaces not covered with sample was done. Then, the first antibody (IgG) was added and incubated overnight. The test materials were washed with Tween - 20 in Tris - buffer solution four times at 3 minutes interval. After washing, the enzyme - lined anti - body (GAR - ALP) was then added and incubated for one hour. Washing was done again to remove unbound antibodies. To produce coloration, the papers were treated with substrate buffer containing nitroblue tetrazolium plus 5 - bromo 4 chloro, 3 - indolyl phosphate. All the procedures stated above were done under room temperature. Bluish - purple coloration which corresponded to positive results was noticed and recorded.

B. Recycling of Antisera :

The same procedure as that in the first trial was followed, but the antisera added to papers have been previously used. Recycling or re - use was done right after the antibodies have been removed from the initial trial. Three recyclings were made.

RESULTS AND DISCUSSIONS

During the blotting process, three of the test material were already eliminated (all mimeographing papers) because they absorbed the sample

(antigen) easily and spread it widely over the surface of the paper.

The initial trial using fresh antisera showed that three of the test materials used gave promising result. These were Cactus Xerox Paper, ordinary paper and Vernal mimeographing paper (Substance 20). These papers produced bluish-purple coloration comparable or the same as that in the NCM. The rest gave no reaction.

The same result was obtained in the first recycling although the degree of coloration was lighter compared to the outcome in the first trial. For the second and third recycling, only the positive control in the nitrocellulose membrane reacted the rest produced no reaction (Table 1).

Table 1: Test materials used compared to nitrocellulose membrane

PAPERS	REACTIONS			
	Fresh	Recycle 1	Recycle 2	Recycle 3
Cactus Xerox Paper	+	+	-	-
Cactus(BP) subs.18	-	-	-	-
Camia (BP) subs.20	-	-	-	-
Cannon(MP) subs.18#				
Advance(MP) subs.20#				
Advance(MP) subs.18#				
Vernal (MP) subs.20	+	+	-	-
Ordinary Xerox paper	+	+	positive control	positive control
Nitrocellulose membrane				

Paper eliminated at blotting stage

BP - Bond Paper

MP - Mimeographing paper

Based on the result of the serological test, 38.63% of the tubers of plants adjacent to the bacterial wilt infected ones were found to be latently infected with the disease.

CONCLUSION

Serological testing for bacterial wilt can now be economized by using locally available material, such as Cactus Xerox Paper, Ordinary Xerox paper and Vernal mimeographing paper (Substance 20), in lieu of nitrocellulose membrane and also by recycling the antisera once. Moreover, gathering of tubers for seed production from adjacent hills of bacterial wilt - infected plants is risky because the degree of latent infection was found to be high with value of 38.63%.

BIBLIOGRAPHY

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