ANTIBIOTIC EFFECTS OF AQUEOUS EXTRACT OF HONEYBEE PROPOLIS AGAINST *XANTHOMONAS VASICOLA* P.V. *MUSACEARUM*.

K.K.M. Fiaboe[1], F. Beed[1], M. Mwangi[2], J. Kubiriba[3] and W. Tushemereirwe[3]

[1]International Institute of Tropical Agriculture (IITA), P.O.Box 7878, Kampala; Uganda; [2]FaCT BioSciences Ltd, P.O. Box 967-00217, Nairobi, Kenya; [3]National Agriculture Research Organization (NARO), P.O.Box 7065; Kampala, Uganda .

INTRODUCTION

Banana bacterial wilt caused by *Xanthomonas vasicola* p.v. *mucasearum* (Xvm) is one of the current most important limitations for banana production in Eastern and Central Africa. The pathogen is mainly spread by insect vectors, tools and planting material. Honey and stingless bees, drosophilids and wasps are the most important insect vectors (Fig. 1). They have been reported to carry the bacteria in and on their bodies. It is not yet known if bee hives serve as pools for Xvm multiplication and spread or if the propolis' antimicrobial defense at hives entrance can eliminate Xvm on honeybee workers' body before entering their hives. Social insects' nests are known to harbor antibiotic protecting systems. Honey bees' hive entrance is squeeze and protected by propolis, serving as protection against various bacteria and fungi. However some microorganisms escape that barrier and induce serious epizootics in bee colonies. The present study aimed at investigating the presence of antibiotic compounds and/or organisms contained in water extract of propolis that can be effective against Xvm**.**

EXTRACTS EFFECT ON XCM GROWTH.

Figure 1: Honey and Stingless bees (A) and wasps (B) visiting male bud

Fresh propolis collected from bee hives and broken into small pieces were then soaked into a sterile-distilled water for a period of 15 days in the incubator at 37ºC. Thereafter, the liquid was filtered twice using a Whatman® No 1 filter paper (185mm diameter). Two types of extract solutions were prepared: a) non sterile propolis aqueous extract solution (NSP) and b) sterile-filtered aqueous extract solution (SP), prepared by filtering again through a Whatman® sterile filter (25mm diameter and 0.2 µm pore size) to remove all living organisms. The control consisted in sterile water kept also for 15 days and filtered as the test extracts. These 3 solutions were then separately mixed with Xvm cell suspension (2 x 106 CFU/ml) at a ratio of 1:4 (v/v, extract/Xvm suspension). An aliquot of 100µL from the mixture was spread plated in 9cm Preti disk previously filled YPGA. For each extract, 20 replications were done, and bacterial growth was evaluated after 72hrs using a scale varying from 0 to 5, based on colony coverage of the medium surface. The scores were set as follow: "0"= no Xvm grown; 1= from 1 colony spot to 20% of Petri dish covered by Xvm colonies; "2"= 20-40%; "3"= 40-60%; "4"= 60-80% and "5"= 80-100% of Petri dish covered by Xvm colonies (Fig. 2).

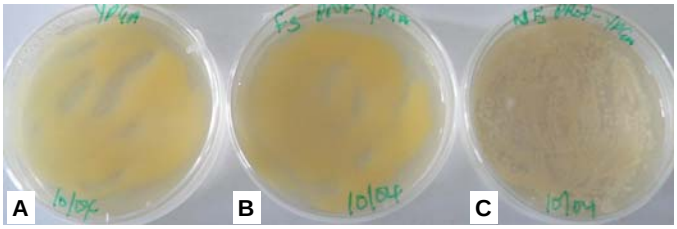

Figure 2: Bacterial growth on YPGA after 72hrs after mixing Bvm with 3 different extracts: A= Sterile water (control); B= Sterili-filtered propolis extract (SP); C= non sterile propolis (NSP).

Growth of Xvm on YPGA was not significantly different between the control and sterilized extract (SP) (mean growth scores of 4.9 ± 0.1 and 4.8 ± 0.1, respectively). However the non sterile extract completely inhibited the growth of Xvm colonies. A, as yet unidentified, gram positive bacteria (PB) was obtained from NSP with mean growth scores of 5.0 ± 0.1.

EFFECT OF PROPOLIS BACTERIA ON XVM

Following results obtained in the previous steps, a pure culture was made from the as yet unidentified gram positive propolis bacteria (PB) in 9cm Petri dished containing YPGA medium. Pure cultures of Xvm were also made in the same medium. In order to test the interaction between both bacteria groups, both PB and Xvm were grown in the same Petri dishes on YPGA media, with eight spots of Xvm (2mm diameter each) alternately surrounded by nine spots of PB (2mm diameter each) (Fig. 3). The control consisted in Petri dishes plated only with either of the 2 bacteria group at the same position and same size as in the treatment. The cultures prepared were let to grow for 72 hours after which scores were attributed to the growth of each group on the medium.

Figure 3: Effect of Propolis bacterium (PB) on *Xanthomonas vasicola* pv. *musacearum*after 72 hrs: mixing PB and Xvm; B= Xvm only

After 72hrs, the mean growth scores were 0.1 ± 0.1 and 4.9 ± 0.1 for Xvm and PB, respectively. The Xvm colonies showed initial convex growth morphologies when adjacent to PB colonies and were then killed.

CONCLUSION

The present results demonstrate the presence of at least one antagonistic bacterial species in the propolis, suggesting possible suppression of Xvm on insect body at hive entrance. These findings warrant further study for possible biocontrol of Xvm.

ACKNOWLEDGEMENTS

- To NaFORRI for availing research field
- To the Dutch government for funding
- To Francis Sebulime for his assistance in data collection