



STUDY THE EXISTENCE OF EBW PATHOGEN IN KOCHO: PLAYS ROLE IN BACTERIAL WILT DISEASE TRANSMISSION

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ABSTRACT

Kocho is a product of matured enset (*Ensete ventricosum*) prepared traditionally by fermenting (for few weeks to some months) a mixture of pulverized pseudostem and com. The experiment was conducted in Hawassa Agricultural Research Centre during 2013 to investigate the existence of pathogen in kocho obtained from infected enset. In the due course of studies the numbers of colonies of pathogen were observed on all the examined samples of kocho. The results indicated that the bacterial wilt pathogen was existed in processed kocho obtained from bacterial wilt infected enset plants. In an early stage of kocho fermentation the average microorganism population was high (221.50 to 151) as compared to the final fermentation stage. The microorganism population was reduced to 35.50 as per fermentation days increased; nevertheless, the exact reasons were not immediately determined. The mean pooled pH of kocho was found at range of between 4.07 and 5.21.

INTRODUCTION

Enset bacterial wilt (EBW) caused by *Xanthomonas campestris* pv. *Musacearum* (Xcm), is an important and the most production limiting constraint in smallholders, causing significant damage and devastating loss to the enset wherever the crop is grown in Ethiopia. The disease is known to attack and kill enset plant at any growth stages, including fully matured (ready for harvest). Once the enset plants are attacked, especially at late maturity stage by the disease, it affects whole systems, and usually a maximum yield loss will occur. Ashagari (1985) reported that a serious outbreak of a bacterial disease occurred with losses up to 70%. The current EBW disease survey (made in some enset fields of the SNNPR), indicated that with individual crop losses of up to 100 per cent was recorded under severe damages (Anonymous 2010).

In the country, the prevalence, severity and distribution levels of EBW disease have a tendency of varying from one enset growing area to the other, depending on various conditions most possibly, farmers' knowledge/awareness, attitudes, clonal diversity and perception towards its management practices. The prevalence of the causal agent of EBW was first reported in Ethiopia by Dagnachew and Bradbury (1968) in very limited enset fields. In the beginning the disease did not draw any attention likely the incidence was not serious as it occurs at present. Lack of knowledge/perception about the nature of pathogen survival, mode of transmission etc. at community level likely contributes to the EBW disease incidence and distributions increasing.

Numerous experiments were conducted to explore the transmissions mechanisms and survival conditions of EBW pathogen. Various workers (Dagnachew and Bradbury 1968; Ashagari 1985; Quimio and Mesfin 1996) have reported that EBW pathogen could survive in the air pockets of enset for a longer time and serve as inoculum for primary infections in the enset fields. Moreover, long distance dissemination of EBW pathogen can also be affected through such mechanism of in-situ survival. According to Gizachew (2000) Xcm is survived in the air pockets or infected leaf petioles and sheaths for about 3 months. Xcm can survive in the soil for about 2 weeks only (Quimio and Mesfin (1996). The pathogen was also found to survive on the surface of contaminated knife for up to 3 and 4 days under humid and dry conditions, respectively (Ashagari 1985).

When EBW disease occurs in enset fields farmers uproot and discard but if the plants are matured or old enough for harvesting, such plants are not discarded but immediately harvested, processed and fermented into kocho (starchy food prepared by fermenting a mixture of the pulverized basal part of pseudostem and underground corm of enset). The time of fermentation of kocho varies from a few weeks to several months depending on enset varieties, temperature, kocho processing experiences etc. (Gashe 1987). However, there is information gap on how long the pathogen survives in the pit and its role in disease transmission. Kocho in Ethiopia is traded across zones and regions. Assuming that the pathogen survives for quite some time in kocho, the role that such trade would play in disease spread is likely great. Therefore, this study is designed to test persistence of the pathogen from

kocho obtained from EBW infected ensets.

MATERIALS AND METHODS

Matured bacterial wilt infected enset plants were collected from the farmers' fields of H/Selam and Bulle woredas/districts. Bacterial wilt diseased plants were uprooted and all leaves (wilted), dead outer leaf sheath and roots from corms were removed before transporting to the Hawassa Experimental Station.

Women having traditionally experienced and who were knowledgeable about the preparation of kocho were selected and provisionally employed to carry out enset/kocho processing following the traditional farmers' practices. The scraped and pulverized masses were thoroughly mixed with small amount of previously fermented kocho (as starter/initiator of fermentation) and placed in pits lined with enset leaves/plastic and left for fermentation at ambient temperature. Kocho was sampled from the pits periodically (15 days interval from the date of processing until final fermentation stage) and the samples were tested for the persistence of the bacterial wilt pathogen. The pH of kocho was also determined in the soil laboratory using pH meter.

Determination of pathogen and pH:

For the bacterial wilt pathogen examination, kocho sample was taken from the pit at 15 days intervals starting from the 1st date of fermentation (0, 15, 30, 45, 60, 75, 90 and 105) up to final fermentation day. Kocho sample was tested by the dilution plate method. About 0.5kg fresh weight of koch sample was collected from the pit and mixed thoroughly, and then from mixed fresh mass 2 gm of sample was taken in 100ml of sterile water and diluted serially. From each dilution one ml quantity of the suspension was added/poured (spread-plated) to petri dishes, to which melted and cooled (45°C) YPSA nutrient media (20 ml) is added and mixed thoroughly with the suspension and then allowed to set. The inoculated petri dishes were incubated at 25 - 30°C for 36 hours after which the appearance of pathogen is observed and colonies were counted using colony counter. For the determination of pH, some amount of fresh kocho was squeezed and liquid/suspension was tested using pH meter.

RESULTS AND DISCUSSION

The experiment was designed to investigate the existence of pathogen from kocho obtained from bacterial wilt infected plants. Kocho is the product of matured enset prepared traditionally by fermenting a mixture of pulverized pseudostem and com. Results of studies indicate that the bacterial wilt pathogen was existed in processed kocho obtained from bacterial wilt infected ensets. In the due course of studies the number of colonies of pathogen were observed on all the examined samples of kocho. In an early stage of kocho fermentation the microorganism population was high as compared to the final fermentation stage. In this study the pooled maximum number of bacteria colonies of 221.50, 153.50 and 151.00 were

recorded on fermentation day 0, 15 and 30 respectively while the colony number of pathogen was low (35.50) on fermentation day 105. The microorganism population was reduced as per fermentation days increased possibly due to destroyed/reduced of appropriate nutrient for pathogen development with increases in fermentation time, although the exact reasons were not immediately determined. The mean pooled pH of kocho was found to be between 4.07 to 5.21 (Table 1).

Fermentation Day	pH			Colony		
	H/Selam	Bulle	Pooled	H/Selam	Bulle	Pooled
0	4.35	4.49	4.42	225	218	221.50
15	4.01	4.12	4.07	183	124	153.50
30	4.12	4.09	4.11	195	107	151.00
45	4.64	4.25	4.45	50	99	74.50
60	4.35	5.16	4.76	43	30	36.50
75	4.76	4.77	4.77	25	26	26.50
90	5.02	4.97	5.00	30	30	30.00
105	5.46	4.95	5.21	40	31	35.50
Average	4.59	4.60	4.59	98.88	91.29	91.00

Table 1:Xcm colonies and pH levels in fermented kocho processed from BW infected enset

In the due course of the studies Xcm colonies and kocho pH levels were corelated to some extent. However, they were not corelated negatively. Relatively at low kocho pH level, the colonies number of Xcm were minimal, as result in this study the effect pH on the colonies development was not significant.

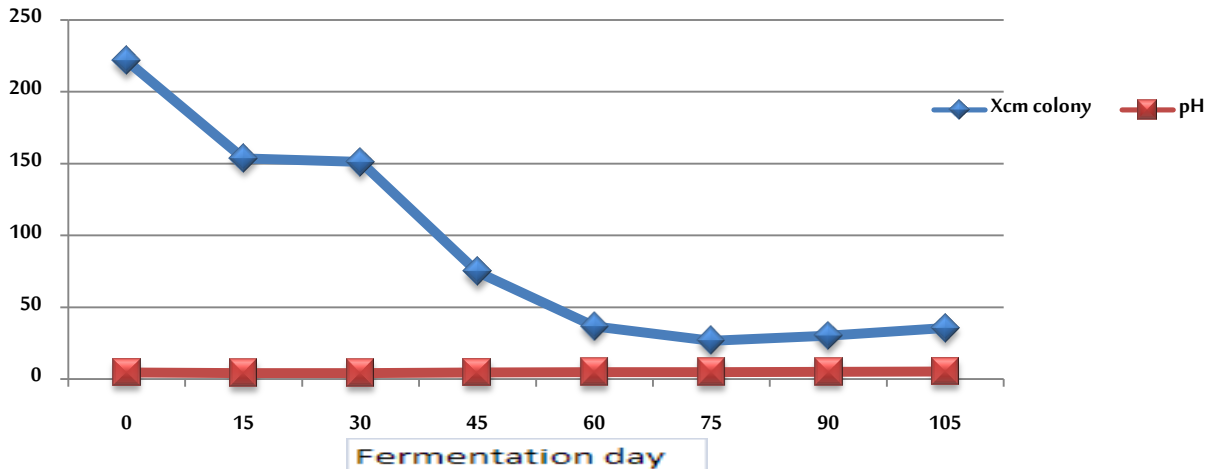


Figure 1: Xcm colonies and pH level in kocho obtained from infeted enset.

Generally, this study indicates that bacterial wilt pathogen was existed in kocho processed from bacterial wilt infected ensets. In all samples of kocho taken from the first fermentation day 0 up to 105 days, the *Xanthomonas* colonies were recorded at varying levels, seeming colonies were independent of pH. These results are accordance with earlier reports of the survival and transmission mechanisms of BW pathogen. The survival and transmission mechanisms of BW pathogen in different substances has bee studied and reported by various authors. According to Quimio and Mesfin (1996) Xcm can survive in the soil for about 3 months, in arid conditions where decomposition of the debris slow. Mwebaze et al. (2006) cited that the survival of Xcm was declined rapidly in non-sterile soil as compared to sterile soil, indicating that Xcm has limited ability to survive saprophytically in soil in the presence of other competing microorganisms. The pathogen was also found to survive on the surface of contaminated knife for up to 3 and 4 days under humid and dry conditions, respectively (Ashagari 1985).

CONCLUSION AND RECOMMENDATION

Farmers uproot and discard when BW disease occurs in thier enset fileds. However, if the plants are matured or old enough for harvesting, such plants are not discarded but immediately harvested, processed and fermented into kocho. In kocho processed from matured BW diseased enset the pathogen survives for quite over three months. As one to three months fermented kocho is ready for use in most of enset farming communities.

Farmers process kocho from matured BW diseased enset for incom generation by selling and/ or for his own family consumption. Some women also pay kocho as a wage for daily labor when workers perform kocho processing or for other tasks (planting, weeding etc.) in the enset fields. In these cases the pathogen can be transmited within the fields and across locations through contaminated kocho. Women process the BW infected enset in the midst of healthy plants. During the cutting and

transporting the infected enset within the healthy plants, the chance of Xcm contamination and transmission is very high. From the present study, the overall results suggest that kocho obtained from BW infected enset is responsible would play role in the disease transmission. As a results, input of this study can be used in the integrated EBW management stratategies, throuhg sensitization of farmers/women not to harvest and utilize the product (kocho) of BW infected enssets.

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