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Risk of banana *Xanthomonas* wilt spread through trade

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Abstract: Banana Xanthomonas wilt is a systemic disease of banana plants. We investigated the risk of spreading *Xanthomonas campestris* pv. *musacearum* (*Xcm*) through asymptomatic mature bunches. Samples of banana fingers and rachis from markets within Kampala, Uganda and at border points of Uganda with DR Congo, Tanzania, Rwanda and Kenya were tested for the presence of *Xcm* through recovery of the bacterium onto semi-selective media. Fingers and rachis infected with *Xcm* were sampled weekly to determine survival duration in such materials. Characteristic colonies of *Xcm* were observed in 89 bunches. Within Kampala, various levels of *Xcm* were detected in the local markets at 21% from Kalerwe, 50% in Nakawa and Nakasera and 53% from Kasubi. At international borders, *Xcm* was detected at 17% in Malaba, 32% at Mutukula, 33% in Busia, 42% at Katuna/Kamwezi, 44% Mpondwe and 62% Mpanga. About 13% of the inoculated plants exhibited symptoms typical of *Xcm* infection. *Xcm* survived for up to six months, with colony counts of 25.3 cfu/gm, 23.1cfu/gm and 20.0 cfu/gm in the peel, pulp and rachis, respectively. This study demonstrated that *Xcm* is carried in traded banana materials over long distances and across borders. The pathogen can survive in the peel and rachis from markets up to 6 months and therefore these organs may act as sources of inocula for new infections. Consquently, there is need to improve on phytosanitary issues to manage spread of *Xcm* and spread of contamination to new areas.

Keywords: Cross-border, markets, Sampling, East and Central Africa

Introduction

Banana (*Musa spp.*) is the fourth most important crop globally with approximately one-third produced in sub-Saharan Africa, where it provides more than 25% of food energy requirements for over 100 million people (FAO, 2006). East and Central Africa (ECA) produce and consume the largest quantities of banana in Africa (Sharrock and Frison, 1999). The types of banana produced and traded include Matooke (cooking), Bogoya (plantain), Apple banana (dessert), Cavendish and Gros michel (Nsabimana *et al*., 2008; Karamura, 1998). Total acreage in ECA under banana ranges between 2-30% with annual per-capita consumption in the range of 28-500 kg (Karamura, 1998). Uganda is a major supplier of bananas to regional markets of Rwanda, Kenya, southern Sudan and Burundi (Spilsbury *et al*., 2004). The largest proportion of banana produced in DR Congo is sold in local markets while an unknown volume is exported to

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Uganda and Rwanda (Spilsbury *et al*., 2004). Only plantain is exported to Uganda from North Kivu, while beer bananas are mainly exported to Rwanda (Spilsbury *et al*., 2002). Bananas are purchased by local and regional traders and agents who deliver them to destination markets at dawn of the following day, using hired trucks. Bananas are transported as bunches or packaged in sisal bags with whorls of pseudostem. The main banana markets in central Kampala are Nakasero, Kalerwe, Nakawa, Balikuddembe and Kasubi. All these markets are supplied by traders who buy bananas from south-western and central Uganda. Common cross border trade routes include Katuna between Rwanda and Uganda; Busia and Malaba between Kenya and Uganda; Mutukula between Tanzania and Uganda and Busunga, Mpanga and Mpondwe between DR Congo and Uganda (Figure 1).

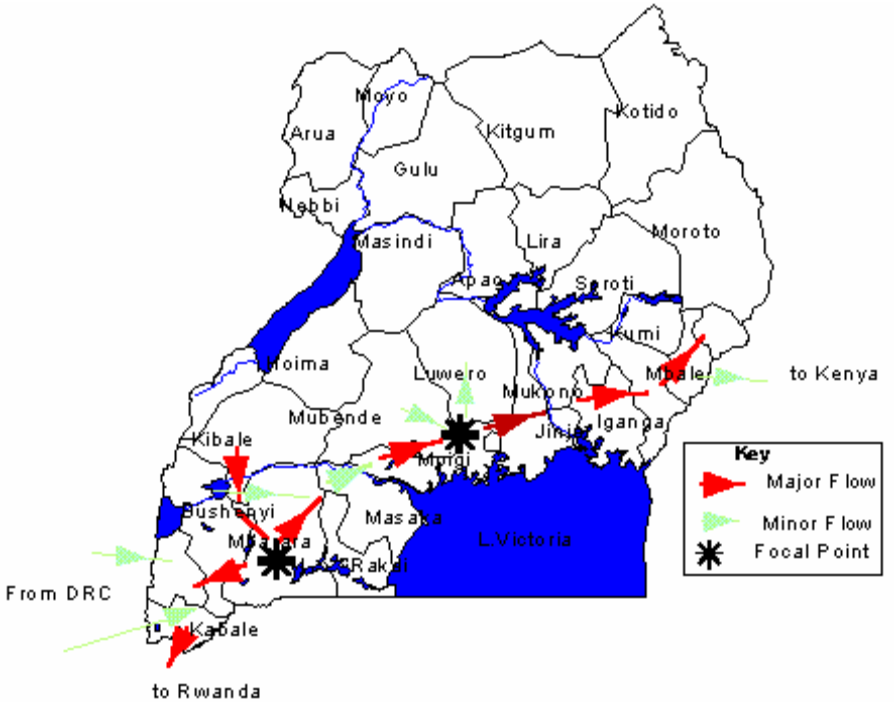

Figure 1 Trade Flow of Bananas within Uganda (Source: Spilsbury *et al*., 2002).

Whereas trade liberalization, globalization and international movement of commodities have increased in the ECA, this may support long distance transport of pathogens. Plant disease outbreaks in the USA and Australia such as Karnal bunt (Bonde *et al*., 1997); Plum pox of stone fruit (Levy *et al*., 2000); Citrus canker (Schubert *et al*., 2001); wheat stripe rust, bacterial blight on cotton, Sugarcane ratoon stunt, potato cyst nematodes, grapevine leaf rust, papaya fruit fly (Alam and Rolfe, 2006) were introduced into these areas through trade.

Xanthomonas campestris pv. *musacearum (Xcm)* the causative agent of Banana Xanthomonas wilt (BXW) is a quarantine pathogen within the ECA region. Pathogen transmission is by insect vectors and by infected planting materials, fruits, plant debris, soil, water and tools (Eden-Green, 2005). Key disease management measures include removing the male bud to reduce insect transmitted infections, disinfecting tools, and using healthy planting material. *Xcm* results in progressive wilting and death of the infected plant. *Xcm* is spread by insects that visit the inflorescence (Fiaboe *et al*.,

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2008) and through infected planting materials and tools that farmers use for practices such as removing dry fibres, older leaves, excess suckers and harvesting bunches and green leaves for domestic use or sale (Addis *et al*., 2008). All banana cultivars are susceptible. Disease management strategies for within field spread exist and are effective (Biruma *et al*., 2007). However the explanation for long distance transport is unknown. One possibility is the transportation via asymptomatic bunches. Plant debris from such infected material could then be sources of primary inoculum for vectors such as bees and other insects which have been implicated in disease spread. The possibility for latent survival of *Xcm* in bananas traded within and outside the country and the ability for the asymptomatic banana parts to cause disease has not been investigated. Our objective was to detect *Xcm* among banana products being traded in major markets and at border points and evaluate the risk of possible spread of BXW into new areas.

Materials and Methods

Diagnosis of the species and pathovar of *Xcm*

Comparative analyses were undertaken to characterize *Xcm*, the causal agent of a wilt of enset and banana, and to assess its relatedness to other xanthomonads by fatty acid methyl esters, genomic fingerprinting using rep-PCR and partial nucleotide sequencing of the gyrase B gene (Aritua *et al*., 2008). The results from all three analyses indicated that strains of *Xcm* are homogeneous and very similar to *X. vasicola* strains isolated from sugarcane and maize from Africa. Pathogenicity studies indicated that strains of *X. vasicola* pv. *holcicola* and *X. vasicola* from sugarcane induced no symptoms on banana, whereas *Xcm* produced severe disease.

Surveys. Surveys were conducted in May, 2008 covering major banana markets in Uganda namely Kalerwe, Kasubi, Nakasero and Nakawa located within central Kampala and border markets of Busia/Malaba (Kenya and Uganda), Katuna/Kamwezi (Rwanda and Uganda), Mutukula (Tanzania and Uganda) and Busunga, Mpanga and Mpondwe markets (DR Congo and Uganda). The areas identified are the major crossing border points that connect Uganda to other ECA countries and the main markets within Kampala city (Figs. 1 and 2).

Sampling procedure. From each of the markets, all banana cultivars traded were targeted. For each cultivar, at least 6 bunches were sampled and from each bunch, samples were collected from the rachis and banana fingers. The banana fingers were picked from the 1st, 2nd and 3rd cluster from the lower, middle and upper parts of the bunch. Finger samples were later divided into peel, pulp and joint samples. Samples of the rachis were also obtained from lines corresponding to clusters 1, 2 and 3 where the banana fingers were picked. The corresponding GPS readings of the market centers that were visited were recorded using (Etrex, Garmin). Truck drivers ferrying bunches were asked from which region the bananas were picked, any sales made enroute Kampala and which other markets they supplied to.

Growth on culture media

Xanthomonas campestris pv. *musacearum* was isolated from the fingers and rachis samples on to Yeast Peptone Glucose Agar (YPGA) (yeast extract 5 g, peptone 5 g, glucose 10 g, and agar 15 g) (Mwangi *et al*., 2007); and plates observed for development of typical yellow, mucoid and convex bacterial colonies characteristic of *Xcm* (Ssekiwoko, 2006). Pure cultures of the bacteria were obtained by streaking on YPGA and incubating for 48 hours at 27-30 ^{o}C.

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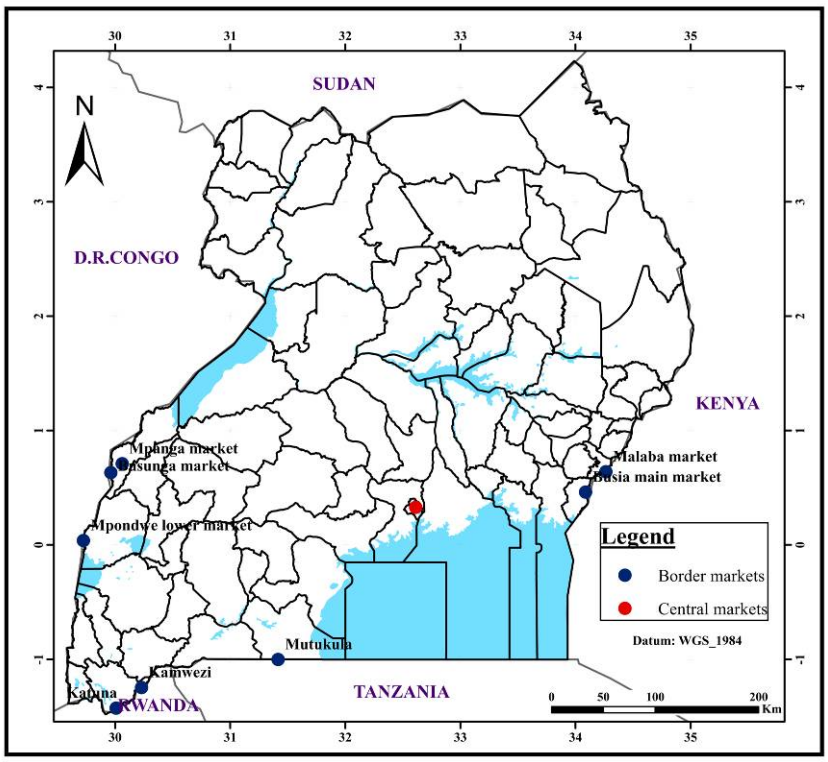

Figure 2 Location of the markets and border points from which samples were picked.

Pathogenicity of recovered cultures

Three month old plants were used to confirm *Xcm* pathogenicity. Inoculum was collected from representative samples of the joint, peel, pulp and rachis. The lamina of the oldest leaf was injected using a syringe fitted with a 28 gauge needle with 1ml (1×10^8 cfu ml^{-1}), OD reading of 0.1 on the spectrophotometer (600 nm). This was repeated for inoculum from each culture recovered from joint, peel, pulp and rachis. The plantlets were observed for onset of BXW chlorotic symptoms on a weekly basis. At 90 days post inoculation, 50 g of fresh tissue from leaves, pseudostem and corm from both asymptomatic and symptomatic plants was ground within a sterile mortar and pestle, serially diluted twice and plated onto YPGA medium to confirm presence of *Xcm*.

Survival of *Xcm* in infected plant parts

This study was done to determine how long *Xcm* remained in peel, pulp, rachis debris after being chopped and if it could act as a source of inoculum for insect vectors and tools to infect healthy plants. The study was carried out at Kifu, Mukono, a banana field found within a forest reserve separated from other banana growing areas. Two banana cultivars, Kayinja and Mbwazirume were used. Infected bunches were harvested and fragmented into different parts (rachis and fingers) and chopped into smaller parts and left on the soil surface for natural influence. On the day of experimental set up as well as on a weekly basis, three replicates of about 50 g of infected rachis and fingers were picked for *Xcm* detection in the laboratory (Mwangi and Bandyopadhay 2006). Colonies characteristic of *Xcm* were counted and expressed as colony forming units (cfu) per dilution per part. The number of bacterial cells per gram of original tissue was then calculated using the formula below:

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Number of bacteria /g of tissue = (Number of cfu × dilution × amount plated × grams of tissue)

The cfu data was subjected to analysis of variance (ANOVA) using Genstat and means were used to plot graphs.

Results

Field observations

Banana sold in the central markets of Kampala and at border areas originated from South Western Uganda in areas of Bushenyi, Ishaka, Mbarara, Fort Portal, Kasese, Isingiro, Ntungamo, Rugaga and Central Uganda from Ssingo and Masaka. Although cooking and dessert bananas are traded within Kampala central markets or exported to neighboring countries, roasting and brewing banana are imported from DRC into Uganda and Rwanda respectively. Many of the traders sell the cooking type with the dessert and roasting sold on a small scale (Table 1).

Table 1 Summary of the Bunches sampled per market per type of banana.

Location	Market name	Cooking banana	Dessert banana	Roasting banana
Kenya-Uganda	Busia	31	0	2
DRC-Uganda	Busunga	15	2	2
Rwanda-Uganda	Katuna / Kamwezi	12	0	0
Kenya-Uganda	Malaba	26	4	0
DRC-Uganda	Mpanga	16	4	6
DRC-Uganda	Mpondwe	18	0	0
Tz-Uganda	Mutukula	30	4	0
Kampala	Kalerwe	13	5	1
Kampala	Kasubi	15	2	2
Kampala	Nakasero	12	0	0
Kampala	Nakawa	7	4	1

Laboratory results

A total of 234 bunches were sampled from the markets and border points (Table 2). Light yellow, circular, high convex colonies typical of Xcm were observed in the rachis, joints peel and pulp (Table 3) totaling to 89 infected bunches after culturing on YPGA accounting for 38% asymptomatic bunches. Of the bunches sampled at international borders, 17% and 33% from Malaba and Busia in eastern Uganda, 37%, 44% and 62% from Busunga, Mpondwe and Mpanga, 42% from Katuna/Kamwezi, (western Uganda), and 32% from Mutukula respectively were contaminated with *Xcm* while in central markets of Kampala, 21% from Kalerwe, 50% from Nakawa and Nakasero and 53% from Kasubi were contaminated (Table 2). For the pathogenicity test, only 13% of the inoculated tissue culture plants exhibited symptoms typical of BXW infection, although *Xcm* was re-isolated from 57% of the plants that did not show any symptoms (Table 4). The leaves of the plants that showed symptoms were drooped towards the apex and plants wilted and died.

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Table 2 Number of samples from selected markets testing positive for *Xcm*.

Location	Market name	Bunches sampled	% samples positive for *Xcm*
Kenya-Uganda	Busia	33	33
DRC-Uganda	Busunga	19	37
Rwanda-Uganda	Katuna / Kamwezi	12	42
Kenya-Uganda	Malaba	30	17
DRC-Uganda	Mpanga	26	62
DRC-Uganda	Mpondwe	18	44
Tanzania-Uganda	Mutukula	34	32
Kampala	Kalerwe	19	21
Kampala	Kasubi	19	53
Kampala	Nakasero	12	50
Kampala	Nakawa	12	50

Table 3 *Xcm detection* in the different samples per market.

Border points / Markets	Joint	Peel	Pulp	Rachis
Busia	0.04c	0.07c	0.06ab	0.03c
Busunga (Bundibugyo)	0.25a	0.18a	0.13a	0.11b
Katuna / Kamwezi	0.06c	0.05c	0.04c	0.05c
Malaba	0.07c	0.04c	0.02c	0.06c
Mpanga (Bundibugyo)	0.23a	0.16ab	0.12a	0.27a
Mpondwe	0.21a	0.21a	0.08ab	0.10b
Mutukula	0.04c	0.07c	0.04c	0.11b
Kalerwe	0.07c	0.07c	0.05c	0.12b
Kasubi	0.12b	0.11b	0.02c	0.15b
Nakasero	0.16b	0.12b	0.05c	0.19a
Nakawa	0.07c	0.02c	0.01c	0.06c

Means across columns followed by the same letter are not significantly different

Table 4 Pathogenicity test of *Xcm* cultures recovered from traded banana samples (finger pulp, finger peel, rachis, and joint) from different varieties at different markets and recovery of viable *Xcm* onto YPGA medium from inoculated plants.

Plant part	Test Variety	Field Characteristic	*Xcm* recovered		
			Leaf	Pseudostem	Corm
Finger	Ndiizi	Asymptomatic	+	-	+
Finger	Ndiizi	Asymptomatic	+	+	-
Finger	Ndiizi	Asymptomatic	+	+	-
Finger	Ndiizi	Asymptomatic	+	-	-
Rachis	Ndiizi	Asymptomatic	-	-	+
Finger	Kayinja	Asymptomatic	-	-	+
Rachis	Kayinja	Asymptomatic	-	-	+
Finger	Nakitembe	Asymptomatic	+	+	+
Finger	Kayinja	Symptomatic	+	+	+
Rachis	Ndiizi	Symptomatic	+	+	+

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Survival of *Xcm* in fingers (pulp and peel) and rachis

Xcm survived in the fingers and rachis for up to 6 months. Significant differences (< 0.001) were observed within the *Xcm* colony populations in the first four months in the different plant parts (Table 5). Noticeable increase and decrease in bacterial concentration were observed to occur overtime with the highest bacterial concentrations recorded within the first month which gradually decreased in the 6th month (Table 5).

Discussion

This study investigated the risk of *Xcm* transmission through banana traded across Uganda. Data from respondents showed that the major route for movement of banana is from Southwest Uganda which is the main banana growing region from where it is transported to Kampala markets and Rwanda through Katuna (Spilsbury *et al*., 2002). The minor route of banana movement is from DRC through Mpondwe and Mpanga into different parts of Uganda and may continue to eastern Uganda into Kenya (Spilsbury *et al*., 2002). The major route defines the movement of East African Highland banana while the minor route describes movement of plantain. There is also limited flow of banana into Tanzania via the Mutukula border since the Bukoba region of Tanzania is also known for its huge production of banana (Mgenzi *et al*., 2006b). The estimated movement of banana along the major and minor routes is over 300 km. The quantity of cooking and roasting banana that is exported and imported by Uganda is unknown since the ministry officials lacked records for this at all border points. Farmers who grow brewing banana are into the beer business and hence do not sell in markets but rather use it to make beer from which they get extra money. Also, priority is given to the cooking banana to supply the urban hub of Kampala whose dwellers do not produce their own food (Spilsbury *et al*., 2002).

Results showed detection of *Xcm* from seemingly healthy bunches from all locations sampled. *Xcm* prevalence levels were higher in samples from DRC-Uganda, Rwanda-Uganda borders and Kampala markets indicating that the Southwestern region still has pockets of infection although they produce enough for the market. Farmers need to remain vigilant in practicing the recommended BXW management control practices (Tushemereirwe *et al*., 2006). Detection of *Xcm* in the bunch implies that disease was not observed in the field at harvesting. The possible route of infection could be through the male and female flower or tools and hence the pseudostem into the vascular system (Mwebaze *et al*., 2006). One of the control options recommended to farmers is to restrict traders from using their own tools to harvest mature bunches from farmer fields as well as disinfection of farm tools during routine management practices (Smith *et al*., 2008).

Results of the pathogenicity test reveal that the *Xcm* isolated from the fingers and rachis is infective although many of the inoculated plants remained asymptomatic implying that the bacteria did not reproduce sufficiently to achieve the threshold required to cause disease. According to Adikini (2010), *Xcm* can survive in banana tissue for periods of over four weeks without showing any external symptoms; however, Tripathi *et al.*, (2007) reported shorter periods for *in-vitro* plants. Results of the reisolation from the leaf, pseudostem and corm indicate that *Xcm* did not move progressively into the pseudostem and corm regions for majority of the test plants. This could have been influenced by a hypersensitive reaction leading to induced resistance by the plant (Klement and Goodman, 1967) or inability of *Xcm* to colonise and multiply within the plant (Agrios, 2005).

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Table 5 *Xantomonas campestirs pv. musacearum* bacterial colony population (cfu/gm) from different parts of bunches.

Plant part	Duration in months					
	1	2	3	4	5	6
Pulp	98.00a	14.28b	8.70cd	6.11de	11.19bc	3.00e
Peel	63.50a	9.27bc	5.89cd	4.14d	11.19b	3.00d
Rachis	28.80a	15.39b	9.85c	7.00cd	11.19bc	3.00d
Mean	12.52	11.78	12.49	9.37	11.19	3.00
Fpr	<.001	<.001	<.001	<.001	NS	NS
%CV	91.30	35.7	44.6	49.3		

Means across columns followed by the same letter are not significantly different.

In addition to being consumed as food or dessert, or processed into wine for the brewing cultivars, products from banana are used for various purposes. Banana peels and the rachis are used to feed tethered animals, mulching to increase soil fertility, while the rachis is used in the steaming of matooke (Reiger, 2006). When used to improve soil fertility, the peels and rachis are discarded into orchards. Survival of *Xcm* in fingers and rachis in this study was for over 5 months. These results are consistent with previous studies on other pathogenic bacteria such as *Pseudomonas phaseolicola* that survive for 20 weeks (5 months) on french bean leaf debris on soil surfaces and 11 weeks (3 month) when debris was buried in soil. This study has revealed that *Xcm* survives much longer (over 5 months) in debris. Farmers could feed banana peeling and rachis debris to their animals rather than discarding it into their plantations and providing foraging bees and other insects with potential innoculum.

Our study shows that the banana bunch harbours latent infections of *Xcm* that could act as sources of inoculum. Since the pathogen remains in fingers and rachis debris for over 5 months it could lead to infection of healthy plants if discarded or used as manure in plantations. Our finding implicates banana trade and provides proof that asymptomatic banana bunches are potential sources of inoculum within and across country borders into disease free areas prompting the need for strong phytosanitary measures at border areas.

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خطر گسترش پژمردگی زانتاموناسی موز از طریق تجارت

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چکیده: پژمردگی زانتوموناسی موز یک بیماری سیستمیک موز می‌باشد. خطر گسترش باکتری *Xanthomonas campestirs* pv. *musacearum (Xcm)* از طریق خوشه‌های بالغ بدون علایم مورد بررسی قرار گرفت. نمونه‌هایی از میوه‌ها و ساقه‌های موز از بازارهای کامپالا، اوگاندا و در نقاط مرزی اوگاندا با جمهوری دموکراتیک کنگو، تانزانیا، رواندا و کنیا برای ردیابی *Xcm* از طریق به‌دست آوردن باکتری روی محیط کشت نیمه انتخابی مورد بررسی قرار گرفت. به‌منظور بررسی مدت بقای باکتری روی میوه‌ها و ساقه‌های آلوده به *Xcm* از این اندامها به‌طور هفتگی نمونه‌برداری شد. کلنی‌های *Xcm* روی ۸۹ خوشه‌ی موز مشاهد ه شد. در کامپالا، سطوح مختلفی از *Xcm* در بازارهای محلی Kalerwe (۲۱٪)، Nakawa (۵۰٪) و Kasubi (۵۳٪) ردیابی شد. در مرزهای بین‌المللی، *Xcm* در Malaba (۱۷٪)، Mutukular (۳۲٪)، Busia (۳۳٪)، Katuna/Kamevezi (۴۲٪)، Mpondo (۴۴٪) و Mpnaga (۶۲٪) ردیابی شد. در حدود ۱۳ درصد از گیاهان مایه‌زنی شده علائم تیپیک آلودگی به *Xcm* را نشان دادند. *Xcm* درون اندامهای مختلف گیاه تا شش ماه زنده می‌ماند. با شمارش کلنی تعداد CFU/gm ۲۵/۲ در پوست CFU/gm ۲۳/۱ در پالپ و ۲۰ در ساقه مشاهده شد. این مطالعه نشان داد که *Xcm* از طریق تجارت موز در سطوح وسیع و در سرتاسر مرزها منتقل شده است. این بیمارگر در پوست و ساقه تا شش ماه زنده مانده و بنابراین این اندام‌های گیاهی به‌عنوان منبع زادمایه برای آلودگی‌های جدید عمل می‌کند. نتیجتاً به‌منظور بهبود وضعیت بهداشت گیاهی و مدیریت صحیح برای جلوگیری از پراکنش باکتری *Xcm* و انتشار آلودگی به مناطق جدید رعایت موارد بهداشت گیاهی ضرورت دارد.

واژگان کلیدی: پژمردگی زانتاموناسی، موز، تجارت، آفریقا

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