

**DIVERSITY OF INSECTS ASSOCIATED WITH BANANA IN BANANA XANTHOMONAS
WILT EPIDEMIC AREAS OF WESTERN KENYA**

ABSTRACT

Banana Xanthomonas Wilt (BXW) caused by "*Xanthomonas campestris* pv. *musacearum*" (Xcm) is a disease that devastates bananas production. The Xcm kills banana plants quickly, spreads rapidly and causes loss of upto 100%. The BXW disease spread on banana occurs mainly through insect vectors which use banana male flower for nectar. A study on banana insect diversity and abundance in epidemic areas of BXW was done in western Kenya. The objective was to determining the diversity of insects associated with banana in BXW epidermic areas in western Kenya including Busia, Kakamega and Siaya counties. A collection of all types of insects visiting inflorecence parts of banana and on the same inflorescence DNA samples were captured by 2minutes dipstick to confirm the presence of Xcm using PCR procedures and eclectroforesis gel picture. Insects were positively identified by entomologist with aid of a dicotomous key and pictures. Significantly a diversified insects were found to be associated with banana in BXW in epidemic areas. The Drosophilidae, Apinae and Tephritidae families were most frequently recorded in banana farms. Bees, grass flies, banana fruit flies, flies, wasps, butterflies, beetles, spiders and black ants formed the diversity. Significantly more bees were recorded followed by flies. Isolates from inflorecence samples, positvely confimed the presence of Xcm through eclectroforesis gel picture in the study

area in western Kenya.

Key words: *Insect frequency, abundance, banana, Xanthomonas wilt, Kenya.*

1. INTRODUCTION

Banana (*Musa* spp.) crop has an annual world production of around 104 million tons in more than 120 countries, of which a third is produced in African, Asia-Pacific, Latin American and Caribbean regions [1, 2]. East Africa is the largest banana-producing and consuming region in Africa with a per capita annual consumption of 250 kgs [3]. In Kenya banana is a major staple crop besides being the most popular eaten fruit, the cooking varieties also represent an important staple food [4, 5].

Banana Xanthomonas Wilt (BXW) devastates banana production. The disease is caused by a bacterium pathogen, "*Xanthomonas campestris* pv. *musacearum*" (Xcm) and was first reported from Ethiopia in the year 1968 [6]. The pathogen kills banana plants quickly and spreads rapidly over a large area making the disease one of the most dreaded in banana (*Musa* spp.). The disease has spread to Uganda, the Democratic Republic of Congo, Rwanda, Burundi, Tanzania and Kenya [7]. In Kenya the disease was first reported in 2006 from Bungoma, Busia, and Teso Counties and later year in Bondo, Siaya, Mumias, Butere, Kakamega and Mt Elgon counties [8]. Banana Xanthomonas Wilt can cause up to 100 percent loss, affecting all types of bananas [9]. Yield losses of up to 70% in beer banana (ABB

group) in central Uganda was reported [10]. Banana Xanthomonas Wilt is transmitted on banana mainly through, insect vectors, contaminated farm tools infected and planting materials which visit male bract and flower scars [11]. Infection of Xcm occurs via inflorescences transmitted by insects under field conditions [7, 12]. Insects are vectors of banana diseases as they collect nectar on the inflorescences or feed on plant parts [13, 14]. Whenever there is a wound Xcm easily ooze out of infected inflorescences and most likely the insects pick up bacteria from these wounds during feeding and or collecting nectar [11, 15]. The study was in western in Kenya where the diseases was widespread by male bud infection. Bees and wasps transmitted *Ralstonia solanacearum* bacteria in Bluggoe plantations [8, 13]. Stingless bees and grass flies were agents of Xcm transmission in banana plantations [16, 17]. The Drosophilidae and Apinae families were most frequently recorded in banana plantations [12, 18]. The objective of the study was to determine the diversity of insects associated with banana in BXW epidemic areas to assist in the integrated disease management.

2. MATERIALS AND METHODS

2.1 Study area

Insects were collected from three counties in western Kenya including: Busia in Agroecological zone Low Midland 1 (LM₁); Kakamega (LM₁) and Siaya (LM₁ and ₂) covering five sub counties, Butere, Butula, Emuhaya, Gem and Ugunja where BXW had been reported. During the study female and male gender households were

interviewed as respondents / owners of banana orchards. A total of 250 farms were **investigated** and at each farm ten samples were collected **randomly** whether BXW affected or not.

2.2 Insect collection procedure

All types of insects **found on** floral parts of banana were collected to **identify** the diversity and abundance of insect floral on banana in BXW epidemic areas. Insects were collected from the male inflorescences using a cotton bag. The collection **including** healthy banana, banana diseased with BXW. An insect net was put around the flower taking care not to disturb the flower and the insects on it. By **grabbing** the net close to its ring the insects were captured in the net. The net was then carefully withdrawn and closed not allow insects to escape out of it. At the bottom of the net, a cotton wool was dropped with Chloroform vapour for 1 minute to knock out the insects to ease their handling. The trapped insects were then carefully emptied in a bottle of alcohol, labelled for further identification.

2.3 Insect identification procedure

A total number of 3,165 insects were collected. Further sorting and identification was done using a stereomicroscope (10-250X) at the **Department** of Plant Science and Crop Protection, Entomology Laboratory at the University of Nairobi, Kenya. The identification was accomplished by an Insect Taxonomist (Entomologist) aided by a dicotomas key and pictures procedures, according to their family, genus and species level. Different insect roles/associations with the banana inflorescence were noted such as; their mode of feeding either sucking or chewing and whether

their characteristics may be linked to the mechanism and mode of spread of the Xcm pathogen. The Objective was to identify the types and abundance of insects associated with spread of BXW above ground; predators, pollinators, herbivores and sap feeders [18]. The hypothesis was that the insect characteristics may be linked to the mechanism and mode of spread of Xcm pathogen.

2.4 Sampling isolates to confirm "*Xanthomonas campestris* pv. *musacearum*"

Two hundred and fifty banana farms surveyed in western Kenya, ten plants were randomly sampled for isolation of BXW pathogen (*Xanthomonas campestris* pv *musacearum*) from inflorescence where the insects were collected. The samples were from different banana cultivars, farmer-managed fields in Busia 100, Kakamega 70, and Siaya 80 samples. A small piece of 2cm² was cut from male flower for PCR amplification, aimed to confirm the presence of Xcm pathogen. The DNA of Xcm was captured using 2minutes dip stick. The samples were taken to International Livestock Research Institute (ILRI). BecA laboratory in Kenya for PCR amplification. The objective was to diagnose and confirm the presence of Xcm pathogen in the study area.

2.5 Extraction of Xcm DNA by Two minutes dipsticks

To capture Xcm DNA, a small cut of 2cm² was made on banana inflorescence and macerated into a bottle containing a 2minutes buffer solution. The bottle was tightly closed then shaken for 30 seconds, after that four dipsticks with the glass fibre-sample pad head were dipped in the bottle for approximately 2 minutes to pick the Xcm DNA. The dipsticks were removed and placed on a clean paper towel to air-dry without exposing them to direct sunlight. Later single of punch 2 mm² disc from the dipsticks eluted in wells for Xcm PCR amplification.

2.6 Procedure and methods used for running the PCR.

PCR master mix ingredients was set up for banana isolates was constituted using a Gene PCR System 9700 amplification. Two primers were used Xcm 38-R (5'CAGCGGCGCCGGTGTATTGAGTG 3') Xcm 38-F (5'CCGCCGGTCGCAATGTGGGTAAT 3') in the premix. The product of the PCR was run on agarose 2% percentage, (100 ml of 1 X TBE buffer plus 2g of Agarose gel) boiled in microwave and cooled then 2ul of gel red was added ran in electrophoresis machine at 120 volts for 30 minutes. This was put into a in UV illuminator for a picture (Tables 1 and 2).

Table 1: PCR master mix ingredients for 2minutes dipstick from a single disc 2mm² for Xcm DNA

Ingredients		Composition
Distilled water	H ₂ O	9ul
Buffer,	KCl	0.5 mM
	Tris-HCl	0.1 mM
	pH	8.3
	MgCL ₂	0.15 mM
	dNTPs	0.25 mM
Taq DNA polymerase		
Primer-Xcm-38F	Primer-Xcm-38F	0.5 ul
Primer-Xcm-38R0	Primer-Xcm-38R	0.5 ul
DNA template	1(2mm ²)disc	1 ul
Total reaction		10ul

Table 2: PCR set up for 2minutes dipstick a single disc 2mm² for Xcm DNA

Step	Temperature	Time	Cycles
Initial denaturing	94 ⁰ C	5minutes	-
Denaturing	94 ⁰ C	20secs	40
Annealing	60 ⁰ C	20 secs	40
Extension	72 ⁰ C	1 minute	40
Final extension	72 ⁰ C	10 minutes	-

2.7 Data analysis

Data on insect diversity were clustered according to; the counties insects were collected from, inflorescence parts of banana from either suspected healthy or diseased banana plants, type of the banana whether dessert or cooking, various banana varieties and topographical elevation the altitude where the crop was grown. The parameters were analysed using Stata (Statistics/Data Analysis) version 12.0. [19]. Mean, standard deviation and standard error were used for comparison.

3. RESULTS

3.1 Insect diversity and frequency

The collected insects comprised of stingless bees (*Apis* sp), grass flies (*Drosophila* sp), banana fruit flies (*Tephritida ceratitis*), black ants (*Plectroctena* sp) and beetles (*Neomyia ruissima*). Stingless bees were the most common insects followed by banana fruit flies, grass flies, beetles and black ants on banana plants. More insects were observed in low altitudes of 1224-1282 meters above sea level (MASL) than medium to high altitude areas. There was an increasing order from Kakamega, Siaya and Busia. Dessert types of banana had significantly more insects than cooking types. Insects observed on fruits were similar to those on flowers. There were similar insects on healthy plants as on those diseased. Stingless bees were frequent insects in dessert banana cultivars as compared to cooking cultivars. Bees had higher mean followed by fruit flies, butterflies. The highest mean number of insects were recorded on 'Ngombe followed by Garisa short, Garisa tall, Gold finger and Valery' while the lowest numbers were found on 'Uganda green' variety (Figure 7). Moderate populations of banana fruit flies observed on varieties; 'FHIA17, Valery and Mysore'. Other insects such as beetles and black ants were few (Figures 1 to 7).

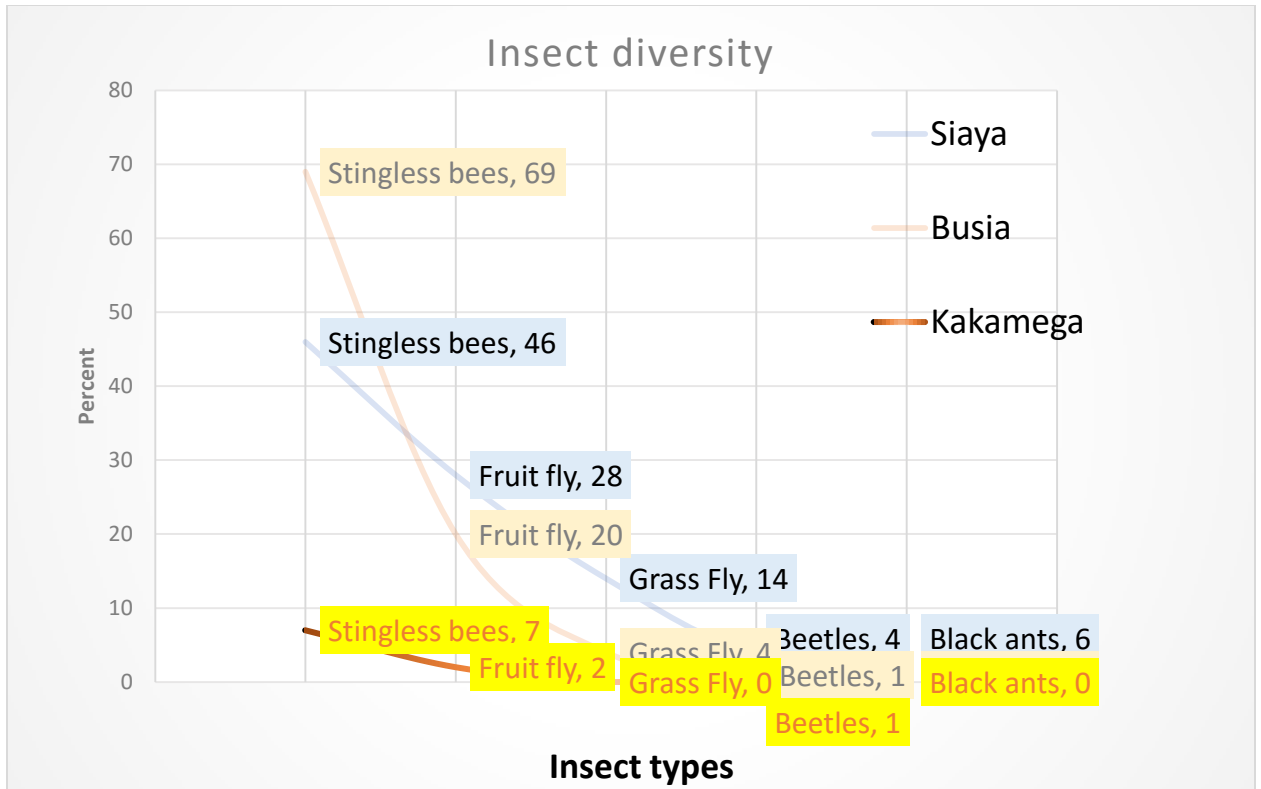


Figure 1: Percent diversity of insects associated with banana plants in BXW epidemic areas in Siaya, Busia and Kakamega counties

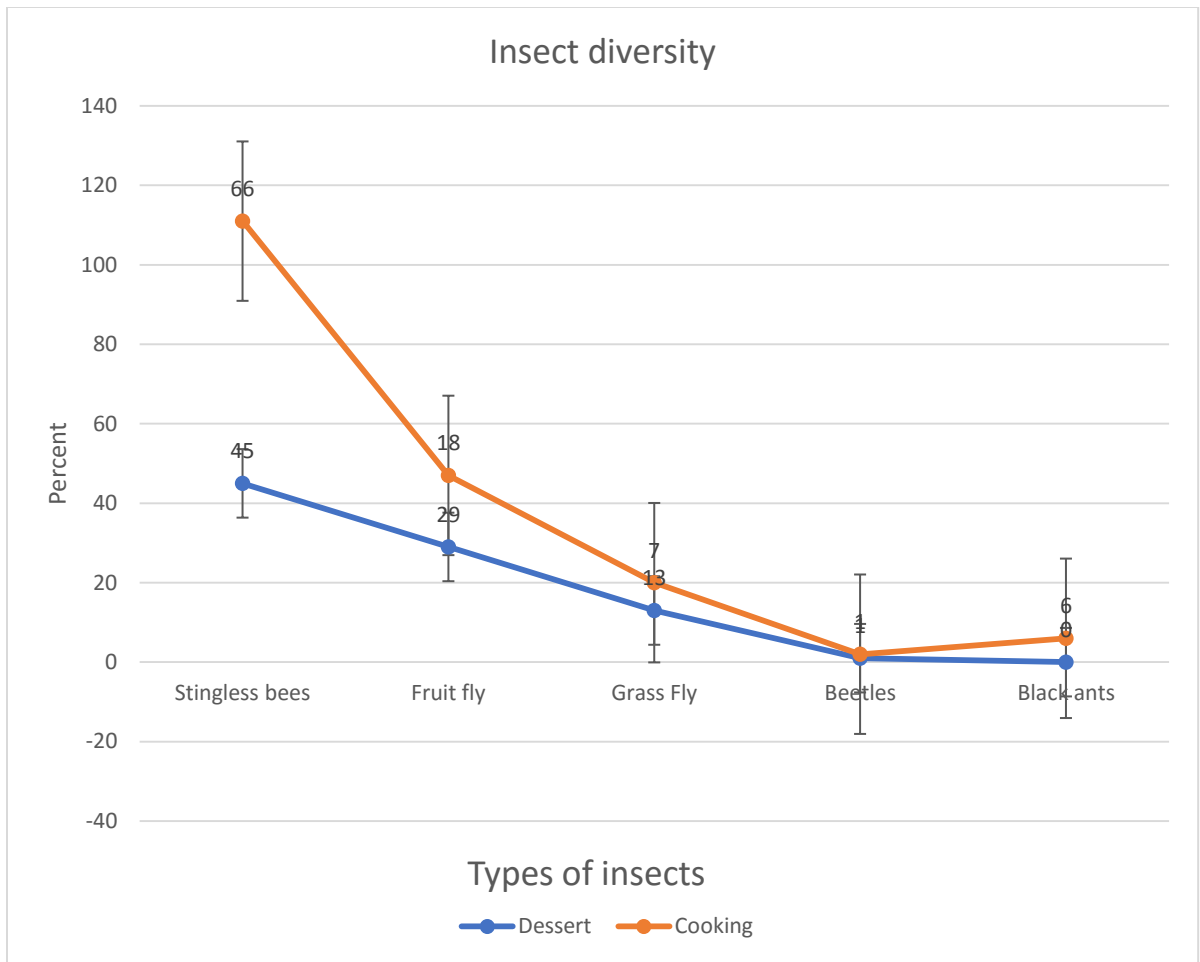


Figure 2: Diversity of insects associated with banana plants in BXW epidemic areas according to banana

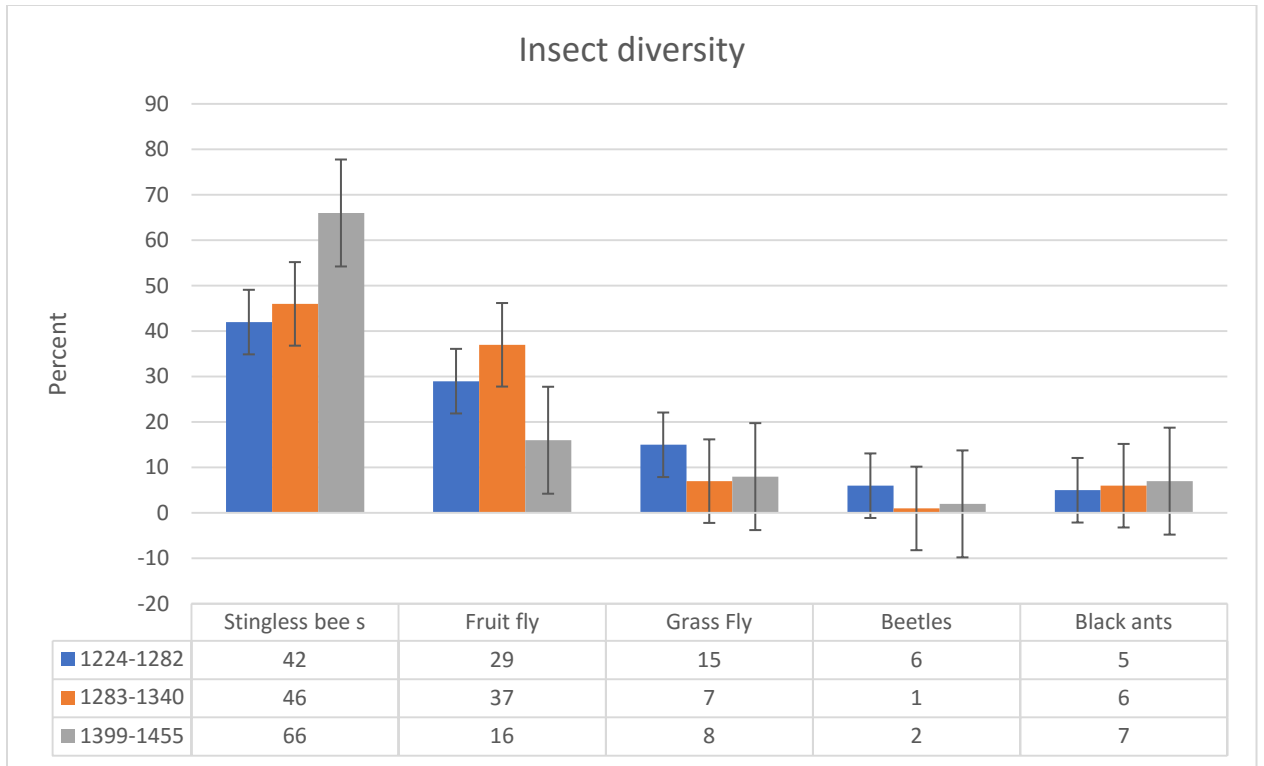


Figure 3: Percent insect diversity according to altitude in BXW epidemic areas.

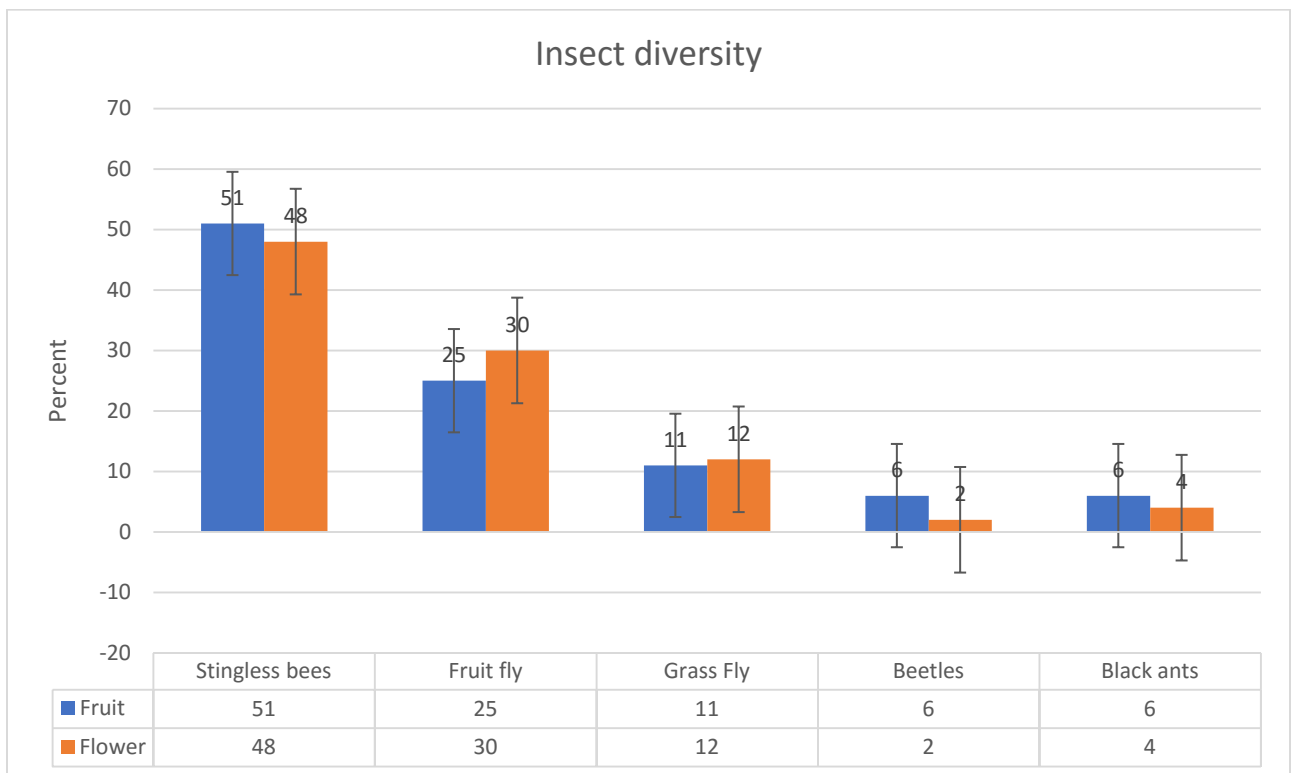


Figure 4: Percent insect diversity from flower or fruit organ of the banana in BXW epidemic areas

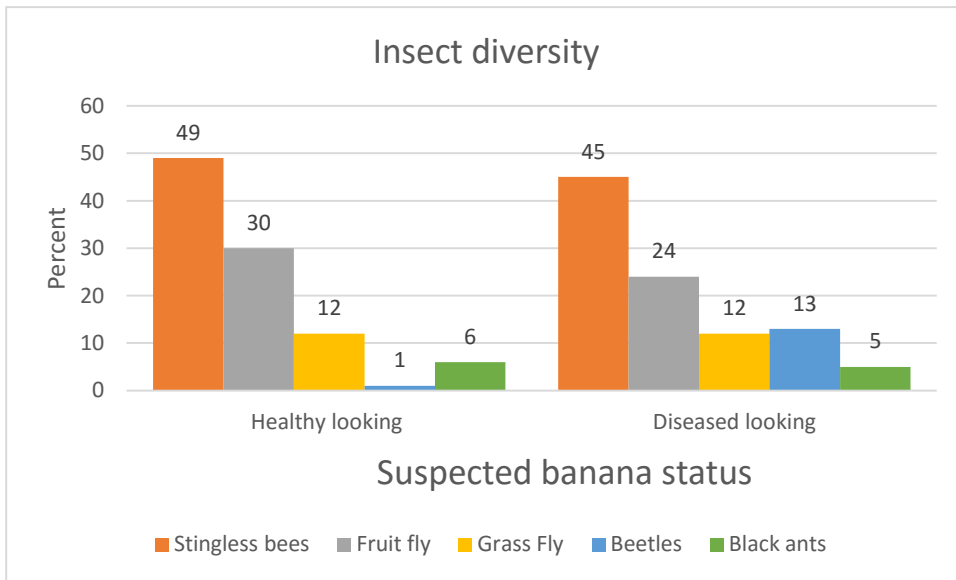


Figure 5: Percent insect diversity according to health status of banana plant in BXW epidemic areas

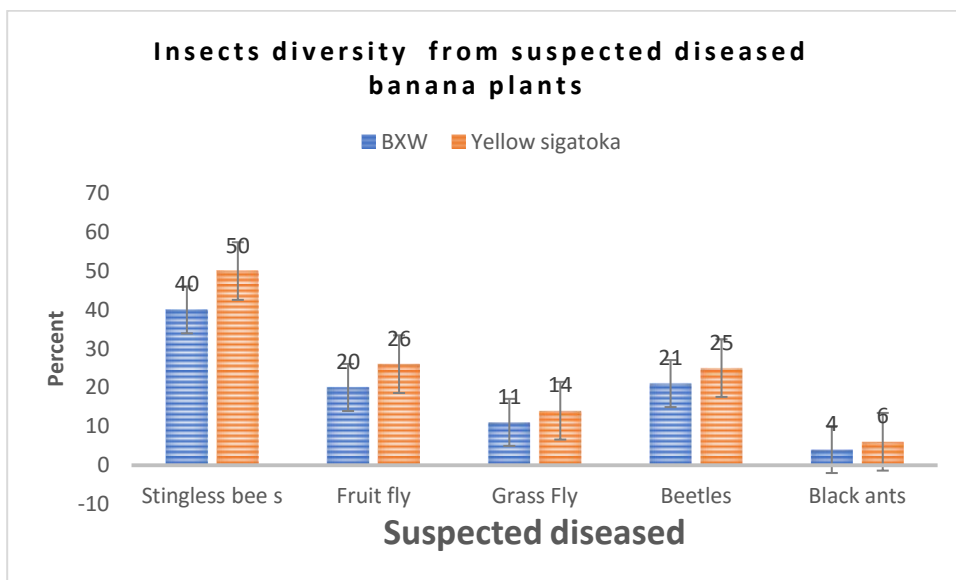


Figure 6: Percent diversity of insects fromr suspected diseased banana plants in BXW epidemic areas

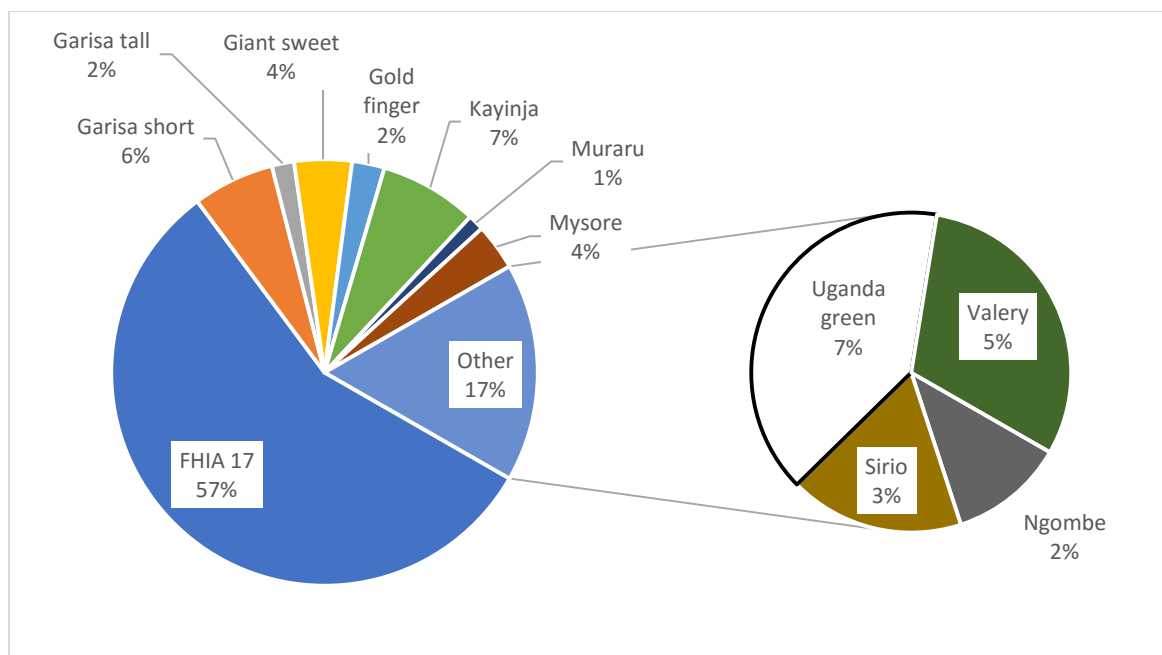


Figure 7: Percent diversity of insects from various banana cultivars in banana *Xanthomonas* wilt epidemic areas.

3. 2 Confirmation of BXW in study areas

Banana *Xanthomonas* wilt disease was confirmed through PCR procedure of collected samples from the studied area (Fig 8 and 9). The results of amplification were positive with a mean percentage of 28%. Busia led with 33%, Siaya 18 and Kakamega 16 (Figure 9). The positive samples produced band with a size of 650 bp that confirmed the presence of *Xanthomonas campestris* pv *musacearum* in studied area (Fig. 8).

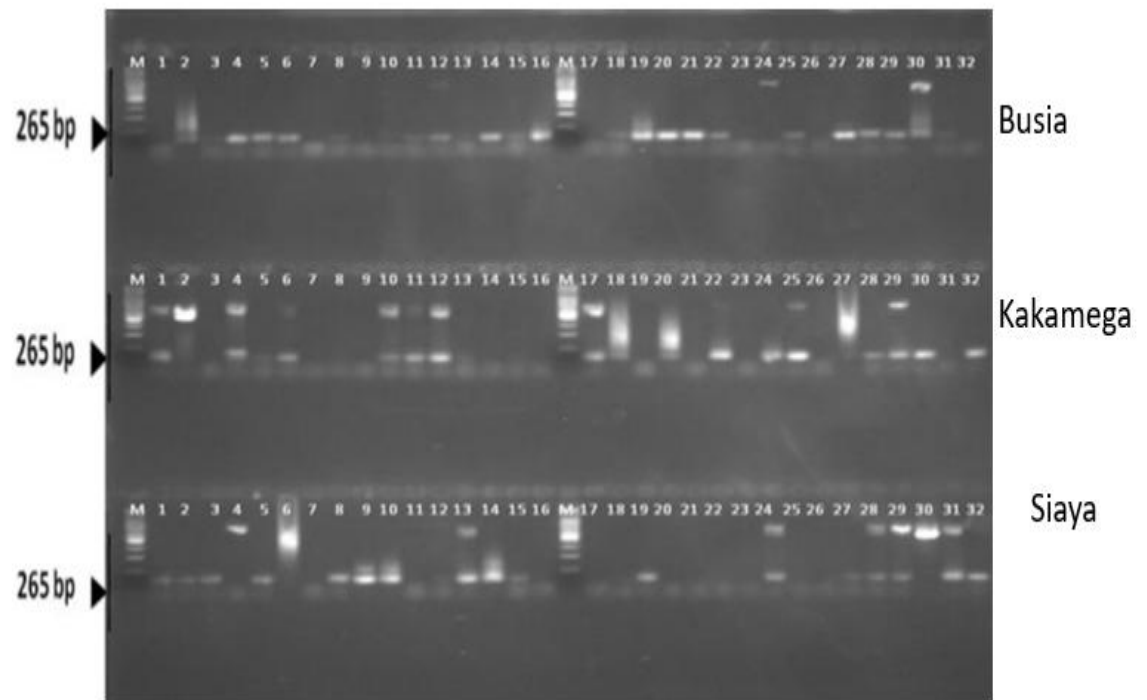


Fig. 8 : Gel images of 2minutes dipstick DNA capture kit isolates from banana inflorescence in Busia, Kakamega and Siaya; at bp=265 Primer base pair, M=100bp Ladder

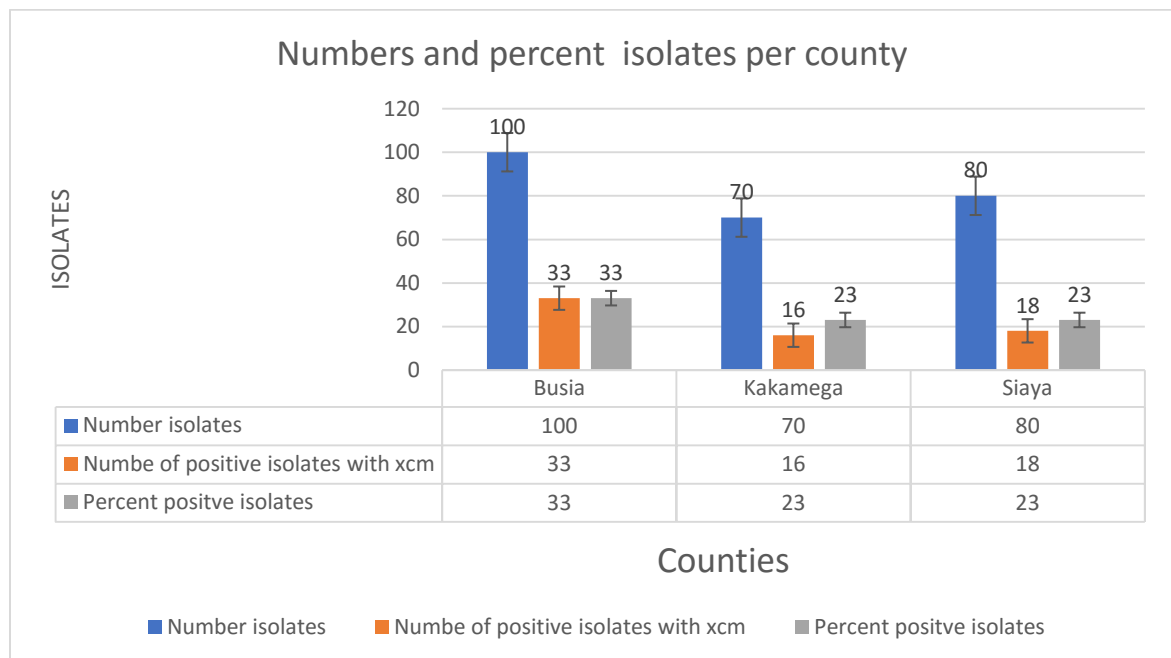


Figure 9: Positive PCR results of Xcm DNA isolates captured by 2 minutes dip stick from Busia, Kakamega and Siaya Counties in BXW epidemic areas

4. DISCUSSION

4.1 Insect diversity and frequency

The types of insects associated with banana plant in BXW epidemic areas were stingless bee, grass fly, beetles, black ants and banana fruit fly. Stingless bees were the most frequent 48% followed by banana fruit flies 27% and grass fly 12%(Figures 1 and 6). These observations agree with studies in Ethiopia and Uganda [16, 18, 20]. The more insects were observed in lower altitude areas of 1224 MASL than in higher altitudes areas (60% of the total populations of insects) and agrees with [21] that more insects population around the lower elevations of Lake Victoria at altitude 1135 MASL than at 1700 MASL.

The study confirmed that there were more insects on the flowers and fruits, due to the fact that they collect nectar as has been reported by other researchers [18, 22, 23]. Significantly more insects were observed in the banana dessert cultivars cavendish group including: Gros Michel, Valery, FHIA 17, Garisa and Gold finger than the cooking type Uganda green in agreement with studies in Ethiopia [18], that Dwarf Cavendish hosted the highest diversity of insect families. Dessert banana had more nectar, produce more pollen and contains higher sugar content thus attracting more insects than the cooking banana [16]. This suggests that disease spread may be faster in dessert banana varieties than cooking varieties. Stingless bees, fruit flies and grass flies were abundant and may be associated with banana inflorescence as they forage for nectar or other symbiotic

relationships. The forage behavior of insects associated with banana flowers may be hypothesized to provide opportunity for picking Xcm from infected plants and transmitting to the non infected plants.

There were diversified insects that were associated with banana plant in BXW in epidemic areas such as bee, grass fly, banana fruit fly, fly, wasp, butterfly beetles, spider and black ants. Bees were recorded as dominant population followed by flies insects due to feed on or collect nector. Conversely some insects like spiders may be concerned with trapping others insects like wasps, ants, flies and moths for their feed and might have no direct activity with banana flowers thus their numbers is low in the inflorescence. The Drosophilidae, Apinae and Tephritidae families were most frequent in banana plantations.

The study area lied at altitude of 1224-1455 MASL. These are with warm climatical conditions and insects diversity and activity are high as confirmed by this study and previous studies [16, 18]. At lower altitude, below 1700 MASL there were more insects than higher altitude and concurs with those reported in Uganda, Democratic Republic of Congo and Ethiopia [16, 18, 21]. There were more insects on the floweres and fruits than on banana pseudostems. Various insects has diffent activities on banana; some collect nector, others feed, make traps for their feed. The banana dessert varieties showed more insects than the cooking varieties and this might be due to the higher sugar content in them as compared to the cooking banana varieties. Dessert bananas may produce more pollen than the cooking varieties and may attribute to more insects attraction than others.

Banana varieties has differences in their floral colours, some has brighter flowers, stronger attractive smell than others and may attract more insects than the dull coloured and less scent varieties. FHIA 17 a dessert banana with a very giant bunch significantly had the highest number of insects, followed by Uganda Green and Kayinja are well adapted in the region [5]. Other varieties, Gross Michel, Valery, Garisa and Gold finger had significantly lower number of insects (Figure 7). Insects act as vectors of Xcm when they contact male bract and flower scars [23], further more infection of Xcm may occurs via infected inflorescences and transmitted by insects under field conditions [7, 16]. Insect vector transmission in banana occurs when bacterial ooze is carried by insects from infected peduncles to the moist cushions peduncle of a healthy plant as they feed on sap oozing from wounds on the inflorescences [13, 24, 25]. Whenever there is a lesion due to Xcm pathogen, easily ooze out of infected inflorescences and most likely insects pick up bacteria from these wounds mechanically, during feeding and or collecting nector [17].

Breaking off the male bud has been recommended to reduce bacterial entry sites in male buds by insects and minimize the Xcm spread from farm to farm [27]. The study confirmed that there were diversified and abundance insects in western Kenya. In banana farms affected by BXW, to eliminate these insects involved in the floral activities it is advategious to practice debudding.

4.2 Confirmation of BXW in epidemic areas of the study

In the study area stingless bees, fruit flies, grass flies, black ants and beetles were present which might be vectors for Xcm [16, 20, 25]. Although insects like bees collect nectar from male flowers of banana they have no pollination role for the formation of the edible banana fruits. Banana crop has its edible fruits formed parthenocarpically ie fruits are formed without pollination [28]. This suggests that insects or pollinators are not required in banana plantations for fruits formation/production. Removal of the male buds reduces insect activities thus reduces chances of Xcm spread [25;29].

Timely debudding, reduces BXW spread in banana farms infected with Xcm and helps in management of BXW [30]. "*Xanthomonas campestris* pv. *musacearum*" were isolated from stingless bees, fruit flies and grass flies that were feeding on male flowers of symptomatic banana plants [16]. These insects causes infection through the moist cushions or scars of recently dehisced banana male flowers and floral bracts [13, 18, 25]. The insects associated with banana inflorescence when they forage for nectar or other symbiotic relationships roles, might likely be vectors. In the study some inflorescence samples were investigated and were found to have Xcm pathogen through PCR procedure (Figures 8 and 9).

5. CONCLUSION

More insect diversity and abundance were observed in the study including stingless bees (*Apis* sp), grass flies (*Drosophila* sp), banana fruit flies (*Tephritida Ceratitis*), , black ants (*Plectroctena* sp) and beetles (*Neomyia ruissima* sp). Bees were the most abundant insects on banana plantation. More insects were observed in lowere altitude areas. *Xanthomonas campestris* pv. *musacearum*" was present in

the study area. Removal of male flower buds reduces insects involvement in floral parts of bananas thus reduces spread of BXW in epidemic areas

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