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Yellow Vein Mosaic disease in kenaf (*Hibiscus cannabinus* l.) under different sowing dates in two agroecologies

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Abstract

Determination of appropriate sowing dates is an important approach towards obtaining optimum crop yield as it affects the resistance/susceptibility of crops to insect pests and diseases. The study investigated the effect of three sowing dates (May, June and July) on the occurrence and incidence of yellow vein mosaic disease in kenaf variety (IFEKEN-100) planted in the experimental fields of the Institute of Agricultural Research and Training (IAR&T) located in Ibadan and Ilora. The incidence of yellow vein mosaic disease was high in May at the two locations with means of 25 and 30% for Ibadan and Ilora, respectively. Plant height was not significantly different in the two locations across the three months. The highest stem diameter was obtained in May from Ilora and Ibadan with means of 1.44 and 1.53 cm, respectively. The best bast fiber yield was recorded in June at Ibadan with a mean value of 1.72 tha-1. Nucleic acid spot hybridization (NASH) was used to confirm the disease and the results revealed that Begomovirus was present in kenaf sowm in the two locations during the period of the three months except in kenaf sown in July at Ilora. The results of this study revealed the importance of sowing dates on the occurrence of viral diseases on the field. If the sowing date is optimum, the effect of viruses may not be pronounced in the crop as seen in the month of June having relatively low virus incidence as well as the highest plant height and bast fibre yield.

Keywords: Begomovirus, kenaf, nucleic acid hybridization, yield

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Introduction

Kenaf (*Hibiscus cannabinus* L.), a member of the family Malvaceae, is the second most important bast fibre crop after jute (Bhaskara et al., 2012). It originated from Africa and members of its genus including roselle (*Hibiscus sabdariffa* L.) are found growing widely in many countries of eastern Africa (Li, 1990; Cheng, 2004). Kenaf is cultivated in more than 20 countries of the world (FAO, 1998). Ninety percent of the sown area and more than 95 % of total production are from China, India and Thailand (FAO, 2003). However, production of kenaf in Africa is very low accounting for only 2.9% of the world production in 2002 (FAO, 2003).

Kenaf is a valuable industrial crop due to its fibre content, medicinal value and effective use in the paper industry (Duke, 1983). Paper made from kenaf fiber is stronger, whiter, long lasting, more resistant to yellowing and has ink adherence better than wood paper (Liu, 2003). The production of kenaf is hindered by many factors among which include the sowing dates, the kenaf variety and pests and diseases.

Proper and suitable sowing date is important in determining optimum yield of crops. Kenaf is susceptible to pests and diseases among which are the following virus diseases: Yellow vein mosaic virus (genus *Begomovirus*) (Chatterjee et al., 2005a), Hibiscus chlorotic ringspot virus (genus *Carmovirus*) (Liang et al., 2002) and Tobacco streak virus (genus *Ilarvirus*) (Bhaskara et al., 2012).

Yellow vein mosaic disease is caused by a Begomovirus. Begomoviruses pose a serious threat to

vegetable and fibre crops in tropical and subtropical countries (Khan, 2000; Boulton, 2003). The genus Begomovirus contains more than 200 species (Fauquet et al., 2008) and belongs to the taxonomic family Geminiviridae. Begomoviruses are characterised by having circular single-stranded DNA genomes encapsidated within twinned isometric particles (Lazarowitz 1992; Briddon and Markham 1994) and are characteristically transmitted by whiteflies (*Bemisia tabaci*). It causes yellowing of veins and veinlets followed by complete chlorosis of the leaves of the affected plants (Chatterjee et al., 2005a).

Studies on the viral diseases especially yellow vein mosaic disease of kenaf have been reported by different authors from different countries (Chatterjee et al. 2005b; Ghosh et al., 2007) but there is a dearth of reports on kenaf viruses in Nigeria. This study was, therefore, carried out to investigate the presence and incidence of yellow vein mosaic disease in kenaf under different planting dates in two agro-ecologies. It is also important to determine the influence of the virus and planting dates on the growth and yield of kenaf fibre.

Materials and Methods

Experimental materials and design

Kenaf variety IFEKEN 100 collected from the Germplasm unit of Kenaf and Jute Improvement Programme, Institute of Agricultural Research and Training (IAR&T), Ibadan was used in this study. The experiment was conducted at the Research farms of IAR&T, Ibadan station ($7^0$38'N, $3^0$84'E 182 masl) and Ilora station ($7^0$81'N, $3^0$82'E 278 masl) in 2016.

The experimental area was laid out in a randomized complete block design with three replicates. The blocks were divided into three plots measuring 3 x 3 m each, representing three planting periods: May, June and July. The plant spacing was 50 x 20 cm and the number of plants/hill was three and later thinned to one plant/hill giving a total plant population of 277 000 plants ha^{-1}.

In each month at 6 weeks after sowing, disease incidence was determined by observing leaves showing the symptoms of yellow vein mosaic disease such as yellow veins and veinlets, mosaic and chlorosis. The symptomatic leaves were counted and expressed as percentage of the total number of plants sampled (Kareem et al., 2016).

Disease Incidence (%) = (Number of symptomatic plants/ Total number of plants) x 100

Five pre-tagged plants were randomly selected for the determination of some agronomic parameters. At 6 weeks after sowing, plant height was determined by using a meter rule to measure the shoot of the plant from the base to the apex in cm. Stem diameter was determined by measuring the diameter of the stem in cm using a venier caliper. The bast fibre was determined by weighing the fibre obtained from the tagged plants after retting and was later extrapolated to tha^{-1}.

Nucleic acid extraction

Symptomatic leaves showing yellow vein mosaic pattern were collected from kenaf plants at six weeks after planting from the two locations. Where yellow vein mosaic disease was not observed, asymptomatic leaf samples were collected. The leaf samples were dried over Calcium chloride until when needed. Nucleic acid extraction kit obtained from Agdia Inc., Elkhart, Indiana, USA was used to extract nucleic acids from the leaf samples using the protocol of Podleckis et al. (1993).

One gram of the dried leaf samples was ground in 1 g of distilled water using mortar and pestle. Approximately 50 µl of the plant sap was transferred into an eppendorff tube containing 450 µl of AG1 Lysis Buffer. The tubes were vortexed for 2 mins and incubated at room temperature for 5 mins. Then the tubes were centrifuged at a maximum speed of 12000 x g for 3 mins. About 300 µl of the supernatant was transferred into a new centrifuge tube containing 350 µl of 100% ethanol. The mixture was vortexed for 2 mins and it was centrifuged (Eppendorf microcentrifuge, 14,200 rpm) at maximum speed for 5 mins.

The supernatant was carefully decanted and the pellet was washed twice in 300 µl of 70% isopropanol. The tubes were inverted on a paper towel and allowed to dry at room temperature for 10 mins. Pellets were later resuspended in 25 µl of nuclease-free water.

Nucleic acid hybridization assay

Approximately 3.0 µl of the extracted nucleic acids were loaded onto a nylon membrane supplied by Agdia's laboratory, USA. The membrane was introduced to labeled probes of Begomovirus in Agdia's laboratory where hybridization of complementary nucleic acid fragment occurred. Chemiluminescent detection was used to produce a visual spot by exposing the membrane to film, indicating a positive result. Negative result did not produce spot on the membrane.

Statistical analysis

The data obtained were analyzed with the Statistical Package for Social Sciencies (SPSS) version 16. The data were subjected to

analysis of variance and means were separated with Duncan's multiple range test at $P \leq 0.05$.

Results

Incidence of Yellow vein mosaic disease in kenaf

Incidence of Yellow vein mosaic disease at Ibadan was high in kenaf sown in May (25%) compared to June (12.3%) and July (16.5%) (Fig. 1). Similarly, In Ilora, the incidence was high when it was sown in May (30%), this was followed by kenaf sown in June (22.7%). However, a very low incidence was recorded in July with a value of 2% (Fig. 1).

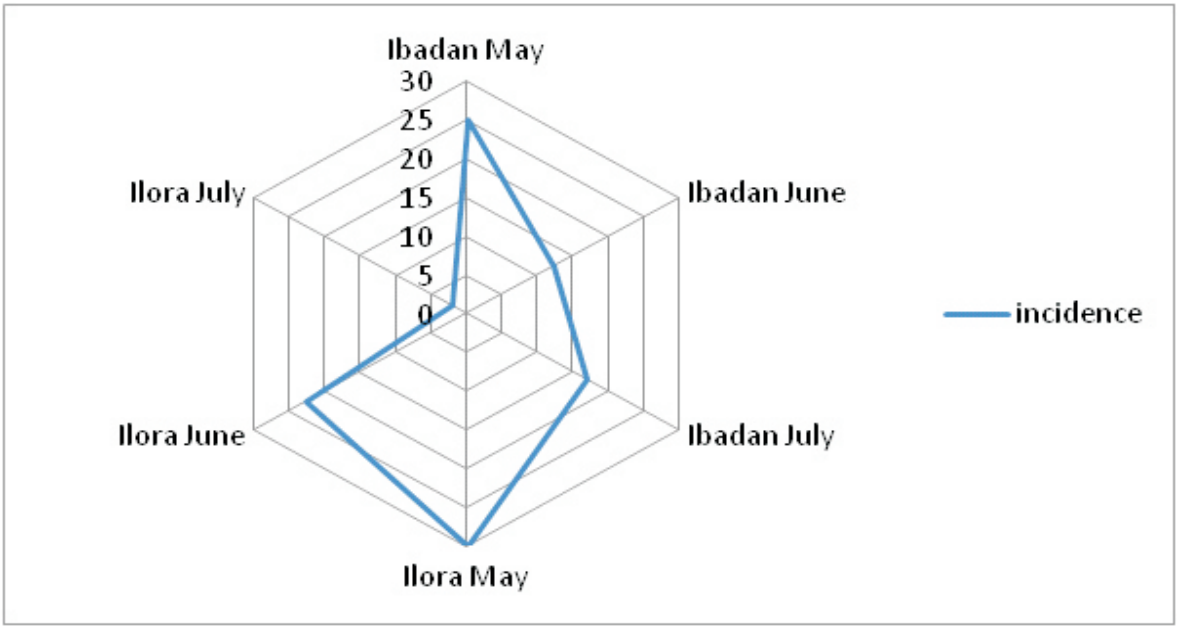

Fig. 1. Incidence of Yellow vein mosaic disease in kenaf

Growth and yield characters

Plant height at Ibadan was not significantly different at $P \leq 0.05$.with mean values ranging from 95.4 to 102.7 cm. Similarly, plant height at Ilora was not significantly different with the mean values ranging from 57.9 and 93.8 cm. The stem diameter of the May planting date at Ibadan and Ilora were significantly higher than the other months with mean values of 1.53 and 1.44 cm, respectively. This was followed by sowing in June at Ibadan station (1.25 cm) (Table 1).

The fibre yield of kenaf at Ibadan was better than that of Ilora. The highest bast fibre yield of 1.7 tha-1 was obtained from Ibadan when the kenaf variety was sown in June while the lowest bast fibre yield of 0.26 tha^{-1} was obtained when the variety was sown in June at Ilora (Table 1).

Table 1. Effect of planting dates on growth and yield characters of kenaf

Location	Month sown	Plant height (cm)	Stem diameter (cm)	Bast fibre yield (tha^{-1})
Ibadan	May	97.2 ^{a}	1.53 ^{a}	1.20 ^{ab}
	June	102.7 ^{a}	1.25 ^{ab}	1.72 ^{a}
	July	95.4 ^{a}	0.98 ^{bc}	1.06 ^{bc}
Ilora	May	91.2 ^{a}	1.44 ^{a}	0.48 ^{cd}
	June	93.8 ^{a}	1.00 ^{bc}	0.26 ^{d}
	July	57.9 ^{a}	0.70 ^{c}	0.53 ^{cd}

Means followed by the same letter along the column are not significantly different according to Duncan Multiple range test at P 0 . 0 5 .

Nucleic acid spot hybridization assay (NASH)

The NASH assay revealed that Begomovirus was present in infected/symptomatic leaves of kenaf with yellow vein mosaic pattern (Fig. 2). All the leaf samples collected at Ibadan from May to July were positive to the complementary probe used in the hybridization assay. At Ilora, positive results for presence of Begomovirus were revealed for May and June planting and a negative result for July planting (Table 2).

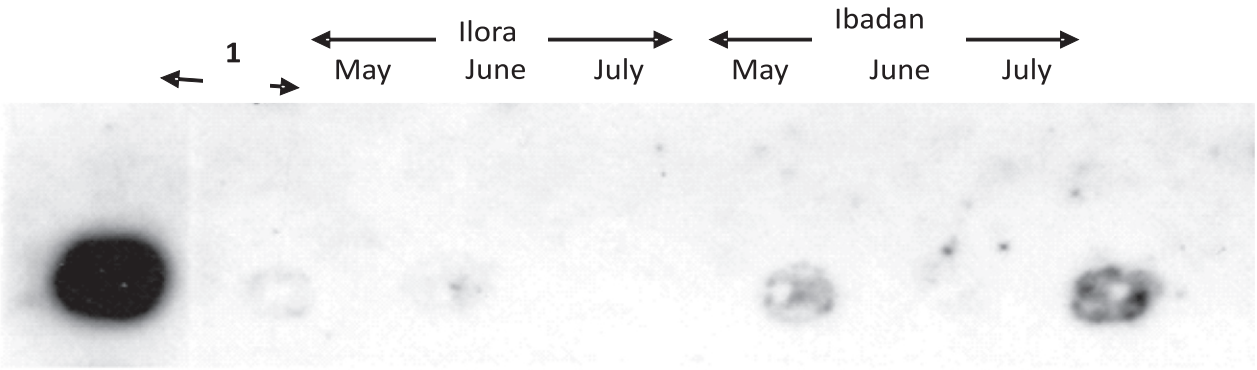

Fig. 2. Nucleic acid spot hybridization (NASH) test of extracted DNA from kenaf leaves showing yellow vein mosaic disease at 6 weeks after sowing. 1 = positive control.

Table 2. Occurrence of yellow vein mosaic disease in kenaf using nucleic acid spot hybridization assay

Month	Ibadan	Ilora
May	+	+
June	+	+
July	+	-

+ = Begomovirus present, - = Begomovirus absent

Discussion

High incidence of Yellow vein mosaic virus was observed in kenaf sown in May at Ibadan and Ilora; this could be as a result of lush growth of weeds and whiteflies during the rainy season. Narula et al. (1999) recorded high incidence of cotton leaf curl disease during the rainy season. Also, the conducive environmental factors which facilitate the build up of whitefly populations during the early crop growth period could be responsible for the high incidence (Roy et al., 2009). The very low incidence in kenaf sown in July at Ilora could be attributed to the fact that the taller plants from the kenaf sown in the previous months i.e. May and June prevented the landing of the Begomovirus vector on the shorter plants. Another reason is that the population of the vector could have reduced as a result of the delay in planting date. Alegbejo (1999) reported in his work that there was reduction in the average number of virus-vectoring beetles caught per plot as a result of delay in sowing date.

Results on plant height and stem diameter showed that early sowing of kenaf apparently led to better growth parameters when compared with late sowing. Ossom and Kunene (2011) reported poor seed emergence and short okra plants as a result of late sowing compared to early sowing. In Ibadan, the kenaf sowed in June produced the best bast fiber yield while the yield of Ilora was very low in the same month. This can be explained by the fact that kenaf sowed in June at Ibadan station had the highest plant height of 102.7 cm which could have contributed to the high yield. Elhag and Ahmed (2014) have reported that good vegetative growth and pod yield means high seed yield. Apart from this, other factors such as environment and climatic conditions can affect crop yield. Sowing date as a factor affecting both plant growth and yield depends on the prevailing environmental conditions especially temperature and relative humidity (Elhag and Ahmed, 2014).

The result obtained from the NASH study revealed the presence of Yellow vein mosaic disease on the kenaf fields. The presence of the virus on kenaf fields is not impossible because kenaf belongs to the Malvacea family which is known to be infected by Begomoviruses. Ghosh et al. (2007) reported the occurrence, distribution and disease incidence of Yellow vein mosaic disease of kenaf in both commercial and experimental farms in India using southern hybridization with Begomovirus specific probe.

The kenaf variety used in this study showed susceptibility to Yellow vein mosaic disease as shown by the NASH test except for the month of July at Ilora which gave a negative result. The positive results could be due to the fact that the population of insect vectors (*Bemicia tabaci* in this case) which transmit viruses is usually high during raining season while the negative result could be attributed to the usual low incidence of the vector, *Bemicia tabaci* responsible for the transmission of Yellow vein mosaic viruses in the month of July. Mastoi et al. (2013) reported that the peak infestation of whitefly in okra varieties was observed in June and pest population started declining on subsequent

observations.

Conclusion

Sowing date is a very important factor that determines the resistance/susceptibility of crops to pests and diseases which directly or indirectly affects the agronomic and yield characters of crops. Selection of appropriate sowing date in a particular year goes a long way in optimizing crop yield. This date should not be fixed over years as a result of changing climatic conditions experienced year in year out. Therefore, the determination of the sowing dates of crops should be a continuous process for yield optimization. Therefore, this study concludes that June is the most appropriate sowing date of kenaf.

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