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INCIDENCE, SEVERITY AND DISTRIBUTION OF YELLOW LEAF CURL DISEASE OF TOMATO IN KENYA

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is an important fruiting vegetable grown in Kenya because of its commercial and high nutritional value. Viruses are a major constraint to tomato production in tropics and sub tropics, eliciting symptoms like stunting, leaf mosaic, distortion, chlorosis, mottling, and vein clearing similar to those caused by abiotic factors. Although begomoviruses are known to cause tomato yellow leaf curl disease (TYLCD) in Kenya, there is limited knowledge on the disease status in tomato fields. The objective of this study was to determine the incidence and distribution of TYLCD in Kenya using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique. A survey was carried out in eight major tomato growing regions (259 fields) in Kenya during September to December, 2018. Presence of tomato yellow leaf curl virus was further confirmed using DAS–ELISA. The disease was present across all the counties surveyed and its prevalence, incidences and severity varied across the counties and among the fields. The mean TYLCD prevalence ranged from 19.5% in Bungoma County, to 64% in Kwale County. There was significant difference ($P<0.05$) in disease incidences among the varieties sampled and the incidence was lower in plants grown from hybrids seed compared to conventional varieties. Mean disease severity was significant ($P<0.05$) and ranged from 0.18 to 2.20. Most farmers planted non-hybrid seeds. There is need for further determination of the diversity of begomoviruses infecting tomato using other techniques to provide more information towards breeding TYLCD-resistant tomato varieties.

Key Words: Begomovirus, DAS-ELISA, *Solanum lycopersicum*, TYLCD

RÉSUMÉ

La tomate (*Solanum lycopersicum* L.) est un important légume-fruit cultivé au Kenya en raison de sa valeur commercial et nutritionnelle élevée. Les virus sont une contrainte majeure à la production de tomates dans les régions tropicales et subtropicales, provoquant des symptômes tels que le

rabougrissement, la mosaïque foliaire, la distorsion, la chlorose, la marbrure, le dégagement des veines similaires à ceux causés par des facteurs abiotiques. Bien que les Begomovirus soient connus pour causer la maladie de l'enroulement des feuilles jaunes de la tomate (TYLCD) au Kenya, les connaissances sur l'état de la maladie dans les champs de tomates sont limitées. L'objectif de cette étude était de déterminer l'incidence et la distribution de TYLCD au Kenya en utilisant la technique de dosage immuno-enzymatique en sandwich à double anticorps (DAS-ELISA). Une enquête a été menée dans huit grandes régions productrices de tomates (259 champs) au Kenya de Septembre à Décembre 2018. La présence du virus de l'enroulement des feuilles jaunes de la tomate a été confirmée par DAS-ELISA. La maladie était présente dans tous les comtés étudiés et sa prévalence, son incidence et sa gravité variaient d'un comté à l'autre et d'une parcelle à l'autre. La prévalence moyenne du TYLCD variait de 19,5 % dans le comté de Bungoma à 64 % dans le comté de Kwale. Il y avait une différence significative ($P<0,05$) dans l'incidence des maladies parmi les variétés échantillonnées et l'incidence était plus faible chez les plantes cultivées à partir de semences hybrides par rapport aux variétés conventionnelles. La gravité moyenne de la maladie était statistiquement significative ($P<0,05$) et variait de 0,18 à 2,20. La plupart des agriculteurs ont planté des semences non hybrides. Il est nécessaire de déterminer davantage la diversité des Begomovirus infectant la tomate en utilisant d'autres techniques pour fournir plus d'informations sur la sélection de variétés de tomates résistantes au TYLCD.

Mots Clés: Begomovirus, DAS-ELISA, *Solanum lycopersicum*, TYLCD

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables in Kenya, cultivated mainly by small holder farmers for commercial and nutritional purposes (Karuku *et al*., 2017). Although the area under tomato production in Kenya has increased over the last decade, yields per unit area remain low (Ochilo *et al.,* 2019). This is attributed to biotic constraints such as insect pests and diseases caused by bacteria, fungi and viruses. Viral diseases have been reported as major limitations to tomato production in Kenya (Macharia *et al.,* 2015; Kimathi *et al.,* 2020; Avedi *et al.,* 2021). Among viruses that cause huge loses in tomato, begomoviruses have become important across the world's tropical and sub-tropical regions. They are vectored by whitefly (*Bemisia tabaci* Gennadius) in a persistent manner, leading to yield losses of up to 100% (Glick *et al*., 2009, Díaz-Pendón *et al.,* 2010). Apart from *B. tabaci*, other studies have identified *Trialeurodes ricini* and *T. vaporarorium* as vectors of tomato yellow leaf curl virus (TYLCV) in Egypt (Idriss *et al.,* 1997) and tomato leaf curl New Delhi virus in India (Sangeetha *et al.,* 2018), respectively.

A number of begomoviruses that cause leaf curl diseases in tomato plants have been reported in Africa. These include tomato yellow leaf curl Mali virus, tomato yellow leaf curl Sudan virus, tomato leaf curl Nigeria virus, tomato leaf curl Uganda virus (ToLCUV), tomato leaf curl Arusha virus (ToLCArV) and tomato leaf curl Tanzania virus (Shih *et al*., 2006; Zhou *et al.,* 2008; Lafeuvre *et al.,* 2010; Kon and Gilbertson, 2012; Kimathi *et al.,* 2020; Avedi *et al.,* 2021). In Kenya, the occurrence of tomato yellow leaf curl disease (TYLCD) caused by TYLCV was first reported on tomato in 1996 (Nono Womdim *et al.,* 2005). However, more recent studies have indicated that tomato plants exhibiting leaf curl disease in Kenya are also infected with ToLCArV, ToLCUV and chickpea chlorotic dwarf virus (CpCDV) (Avedi *et al.,* 2020; Kimathi *et al*., 2020; Avedi *et al.,* 2021). The increasing significance of leaf curl diseases in agriculture has led to scientific interests in studying the biology and transmission of associated begomoviruses (Fiallo-Olivé *et al*., 2020).

The spread of diseases caused by begomoviruses to new geographical areas is occasioned by intensified cropping systems, movement of infected plant material and geographical expansion of vectors through international trade (Kanakala and Ghanim, 2020). The emergence of new begomoviruses with increased host range, virulence and transmission has also been reported (Moriones and Navas-Castilo, 2008). Additionally, occurrence of mixed inter- and intra-begomovirus species infections in agricultural crops worldwide do occur; while synergistic interactions between these species lead to emergence of hyper-virulent recombinants (Silva *et al.,* 2014; Kanakala and Ghanim, 2020). The objective of this study was to determine the incidence and distribution of TYLCD in Kenya using the double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) technique.

MATERIALS AND METHODS

Study sites. A field survey was conducted in major tomato growing counties in Kenya, namely Bungoma, Taita Taveta, Meru, Kwale, Nakuru, Baringo, Kirinyaga and Kajiado (Fig. 1), during September and December, 2018. The counties are characterised by tropical climate, with different agro-ecological zones and variations in rainfall and temperature: an annual bimodal rainfall pattern of between 800 and 1400 mm and temperatures ranging from 15 to 30 °C with well-drained loam soils.

Yellow leaf curl disease sampling. A combination of purposive and simple random sampling was used to select tomato farms and sites for this purpose. Fields were sampled by stopping at regular pre-determined intervals of 3-8 Km along major and feeder roads that traversed each sampling area. The number of fields surveyed per county depended on the availability of tomato farms at the time of survey. One to three months old tomato crops were assessed for TYLCD symptoms. In each field, scoring for yellow leaf curl disease symptoms was done on 30 tomato plants, with assessments made along a X-shaped transect extending from the four corners of each field. Along the transects, five symptomatic samples were collected and where no symptoms were observed, five asymptomatic plants were also picked. Details of the tomato variety planted on the fields were also recorded. Moisture on the leaves were blotted out with absorbent paper and the sample dehydrated over anhydrous Calcium chloride ($CaCl_2$) placed in paper bags. The samples were carried to the Plant Quarantine and Biosecurity Station laboratory - Muguga (location) to confirm virus presence using DAS-ELISA. Geographical coordinates at each sampling site were taken using a global positioning system device (Magellan Triton 'Windows CE Core 5.0 X11-15302).

TYLCD prevalence, incidence and severity. Disease prevalence was assessed by determining the number of fields where TYLCD was recorded in relation to the number of fields sampled in different counties. The disease incidence was visually determined as the percentage ratio of the number of symptomatic plants against the total plant population in the area assessed (Nono Womdim *et al.,* 2005). Disease severity on the other hand, was determined by evaluating the percentage of leaf areas infected against a five point scale, such that 1 = 1-20% (very mild), 2 = 21- 40% (mild), 3 = 41-60% (severe), 4 = 61-80% (very severe), and 5 = 81-100% (almost dead) as described by Mwangi *et al*. (2015).

Serological detection of TYLCV. All tomato leaf samples collected were tested for the presence of TYLCV by DAS-ELISA, as described by Clark and Adams (1977). The antisera and controls were purchased from Agdia® USA and all buffers were prepared according to the specifications provided by the manufacturer. Extraction buffer was prepared in the laboratory using 0.2% chicken egg albumin, 0.13% sodium sulfite, and 2%

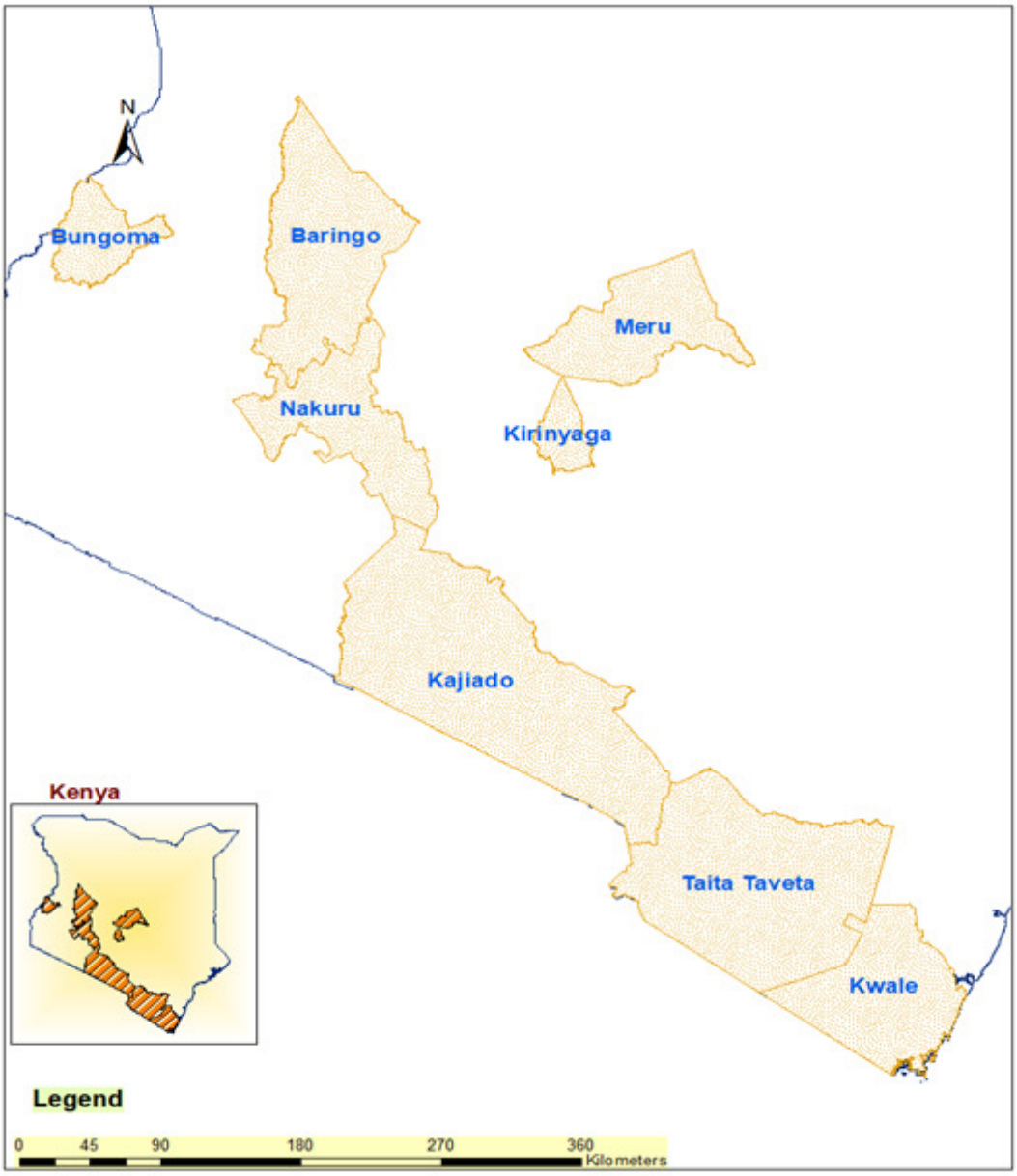

Figure 1. Map of Kenya showing counties where tomato leaf samples were collected.

polyvinylpyrrolidone (Sigma-Aldrich, St. Louis, MO) in 1× phosphate-buffered saline with Tween-20 (PBST). Each sample was crushed in 10 ml of extraction buffer using a mortar and pestle; and incubated at 37 °C for 4 hours. Each well within the micro-titre polystyrene plates (F96 Maxisorp, Nunc, Thermo FisherScientific, Waltham, MA) was coated with 200 µl of TYLCV-specific antibodies, diluted at 1:200 in coating buffer (0.015 M Na_2CO_3, 0.0349 M $NaHCO_3$, pH 9.6) followed by incubation at 30 °C for 4 hours. The plates were later washed thrice in 1×PBST (phosphate buffered salineTween-20: 137.00 mM NaCl, 1.46 mM KH_2PO_4, 7.75 mM Na_2HPO_4, 2.68 mM KCl, pH 7.4, 0.05% (v/v)

Tween-20) and dried on blotting paper. Two hundred microliters of the extracted sap from each test sample were transferred per duplicate wells onto the micro-titer plates and incubated at 4 °C over night. Plates were again washed three times with 1x PBST, blotted on blotting paper and enzyme conjugate (200 µl), diluted in enhanced chemiluminescence buffer at 1:200 (v/v) were added to each well. Plates were subsequently incubated at 37 °C for 3 hours and washed thrice. Two hundred microliters of freshly prepared substrate (1 mg ml^{-1} p-nitrophenyl-phosphate in substrate buffer + 10% Diethyl ethanolamine, pH 9.8) was added to each well and incubated at 37 °C for 60 minutes. Within the micro-titre plates, positive and negative controls were included in duplicates.

Plates were then assessed visually for colour change and absorbance measured at 405 nm wave length, using a BIO-TEK®micro-titre plate reader Model EL×800(BIO-TEK Laboratories, Winooski, Vermont, USA). All samples were assayed in duplicate and the results inferred to be positive if the absorbance was greater than or equal to twice the average readings of the healthy controls.

Data analysis. Data were analysed through descriptive statistics (frequencies, percentages and mean values) for all continuous variables to generate tables, and analysis of variance was carried out using SAS version 9.1 (SAS Institute, 2004) at $P<0.05$ significance level. The differences between means was determined using Fischer's Protected LSD at P = 5%.

RESULTS

Tomato Yellow Leaf Curl Disease prevalence, incidence and severity. The symptoms of TYLCD observed in the fields were mainly stunting, chlorosis, flower abscission, upward leaf curling and reduced leaved sizes (Fig. 2). These were present across all the counties surveyed. A total of 1,275 plant samples were collected from 259 fields in the 8 counties. The TYLCD prevalence, incidences and severity varied intra- and inter-county wise. Mean disease prevalence was 53.06% as observed across the eight sampled and ranged from 19.5% in Bungoma to 64.0% in Kwale (Table 1). The TYLCD incidence varied with significant difference (P=0.001) across the counties. The mean disease incidence ranged from 0.3% in Bungoma to 38.8% in Kwale with Baringo and Kirinyaga recording incidence of 34.2% and 23.7%, respectively.

Figure 2. Tomato plants exhibiting TYLCD symptoms.

TABLE 1. Disease prevalence, incidence and severity across the sampled counties

County	Number of fields surveyed	Prevalence (%)	Mean incidence (%) +SEM (field)	Mean incidence (%) (ELISA)	Mean severity +SEM
Nakuru	55	57.5	$22.9^{bc} \pm 2.5$	$28.3^{bc} \pm 3.7$	$1.73^{abc} \pm 0.14$
Kirinyaga	60	57.0	$23.7^{bc} \pm 3.2$	$31.4^{bc} \pm 9.3$	$1.62^{abcd} \pm 0.15$
Meru	19	47.0	$18.6^{c} \pm 3.7$	$22.6^{c} \pm 7.2$	$1.67^{abcd} \pm 0.22$
Bungoma	41	19.5	$0.3^{d} \pm 0.1$	$1.7^{e} \pm 0.4$	$0.18^{e} \pm 0.07$
Taita Taveta	39	61.5	$16.7^{c} \pm 3.4$	$18.5^{c} \pm 5.8$	$1.30^{bd} \pm 0.17$
Kajiado	19	58.0	$14.8^{c} \pm 4.4$	$17.4^{d} \pm 3.2$	$1.37^{bcd} \pm 0.29$
Kwale	11	64.0	$38.8^{a} \pm 10.0$	$41.4^{a} \pm 8.4$	$1.85^{ab} \pm 0.38$
Baringo	15	60.0	$34.2^{ab} \pm 8.4$	$33.6^{ab} \pm 6.3$	$2.20^{a} \pm 0.35$
P value (5%)			<0.001	<0.001	<0.001
LSD			11.87	13.04	0.6011

Values followed by same letter within the column are not significantly ($P < 0.05$) different. SEM = Standard Error of Means

Based on the serological detection, Kwale County had the highest disease incidence (41.4%), followed by Baringo (33.6%); while Bungoma County had the least (1.7%). Field assessment of the TYLCD incidence levels were consistently lower than that those based on serological assays (Table 1). The TYLCD disease severity was also statistically different ($P<0.001$) across the sampled counties and ranged from 0.18 in Bungoma to Baringo with 2.20. Most counties had scores of <2.00, which indicated very mild infections (Table 1). TYLCD among the most cultivated tomato varieties

Tomato varieties planted by farmers sampled during the survey included Riogrande, Kilele F1, Onyx F1, Elgon, Cal J, Safari, Shanty F1, New Fortune maker, Eden F1, Nyati F1, Asila F1, Money maker, and Pesa F1. The varieties being cultivated across the farms varied between and within the counties. Some tomato farmers (20.5%) across all counties preferred variety Riogrande (Table 2). However, more than one variety was grown across the counties; while in other fields, several varieties were grown simultaneously.

A few farmers (3.5%) planted recycled seeds from the previous crop harvest. The incidence of TYLCD among the varieties sampled was statistically different ($P=0.001$) and ranged from 0.4 to 59.1% (Table 2). Recycled seed had the highest disease incidence (59.1%), followed by 'New Fortune maker' (36.9%); while 'Onyx F1' had the least score (0.4%).

Generally, disease incidence was lower in hybrid varieties compared to conventional cultivars (Table 2). Similarly, there were significance differences($P=0.001$) between the mean disease severity amongst the varieties. The severities ranged from 0.18 to 3.05 with 'New fortune maker' having the highest disease severity (3.05); while 'Onyx F1' had the least (0.18) (Table 2).

Category of tomato seeds planted in farmer fields across the sampled region. Data collected on type of seed planted was classified into hybrids, non-hybrid (conventional seed) and recycled seed. It was observed that 53.63, 42.63 and 3.75% of the farms planted non-hybrid, hybrid and recycled seeds, respectively. 'Rio Grande' was the most cultivated variety (20.5%), followed by 'Kilele F1' (8.9%) (Table 3). The use of non-hybrid seed ranged from 32 to 69% across the

TABLE 2. Incidence and severity of TYLCD on tomato cultivars in Kenya

Variety	Number of farms	Mean TYLCD incidence +SE	Mean TYLCD severity + SE
Recycled	9 (3.5%)	$59.1^{a} \pm 3.4$	$3.04^{ab} \pm 0.11$
New fortune maker	13 (5.0%)	$36.9^{b} \pm 3.0$	$3.05^{a} \pm 0.15$
Riogrande	53 (20.5%)	$33.7^{bc} \pm 2.6$	$2.08^{acdef} \pm 0.14$
Money maker	14 (5.4%)	$33.1^{bc} \pm 5.9$	$2.15^{acdef} \pm 0.30$
Cal J	15 (5.8%)	$29.4^{bcd} \pm 5.1$	$1.94^{acdef} \pm 0.25$
Tecsim	5 (2%)	$19.7^{bcde} \pm 0.0$	$2.10^{abcde} \pm 0.00$
ATM	11 (4.2%)	$13.8^{bde} \pm 5.3$	$1.70^{acdefg} \pm 0.45$
Nyati F1	7 (2.7%)	$13.7^{bde} \pm 10.5$	$1.47^{cdefgh} \pm 0.22$
Safari	10 (3.9%)	$12.5^{ef} \pm 3.4$	$0.99^{ceghij} \pm 0.20$
DRD	11 (4.2%)	$10.5^{bdefg} \pm 7.3$	$1.50^{acdefgh} \pm 0.30$
Star F1	9 (3.5%)	$9.3^{bcdefg} \pm 0.0$	$2.30^{abc} \pm 0.00$
Big Rock F1	12 (4.6%)	$5.7^{efg} \pm 2.9$	$1.03^{ceghi} \pm 0.22$
Pesa F1	7 (2.7%)	$5.0^{efg} \pm 2.8$	$1.00^{ceghij} \pm 0.31$
Eden F1	12 (4.6%)	$4.1^{efg} \pm 1.6$	$0.84^{ceghijkl} \pm 0.27$
Kilele F1	23 (8.9%)	$3.5^{eg} \pm 0.8$	$0.71^{cehijkl} \pm 0.10$
Shanti F1	8 (3.1%)	$3.4^{efg} \pm 2.1$	$0.54^{ehijklm} \pm 0.14$
Assila F1	13 (5.0)%	$1.6^{eg} \pm 0.6$	$0.53^{ehijklm} \pm 0.19$
Rambo F1	11(4.2%)	$0.7^{eg} \pm 0.7$	$0.37^{ehijklm} \pm 0.37$
Onyx F1	16 (6.2%)	$0.4^{eg} \pm 0.2$	$0.18^{ikm} \pm 0.10$
LSD		19.27	1.109

Values followed by same letter within the column are not significantly ($P < 0.05$) different. SEM-Standard Error of Means

TABLE 3. Occurrence of TYLCD within tomato fields by seed type across the surveyed counties in Kenya

County	Number of fields surveyed	TYLCD Prevalence (%)	Mean number of farmers using hybrid seed (%)	Mean number of farmers using conventional hybrid seed (%)	Mean number of farmers using recycled seed (%)
Nakuru	55	57.5	53	47	0
Kirinyaga	60	57	57	37	6
Meru	19	47	30	69	1
Bungoma	41	19.5	68	32	0
Taita Taveta	39	61.5	43	52	5
Kajiado	19	58	41	56	3
Kwale	11	64	22	67	11
Baringo	15	60	27	69	4
			42.625	53.625	3.75

counties with a mean of 53.63%. Baringo County had the highest number of farmers who planted conventional seed (69%), followed by Kwale (67%); while Bungoma had the least (32%). The use of hybrid seed across the counties ranged from 22 to 68%; with Bungoma having the highest (68%) and Kwale the least (22%); with an overall mean of 42.63% across all the counties. The proportion of farmers that planted recycled seed ranged from 0 to 11%, with an average of 3.75%. Kwale was highest in recycling (11%); while Bungoma and Nakuru were the least (0).

DISCUSSION

Tomato Yellow Leaf Curl Disease prevalence, incidence and severity. The variation of disease incidence and severity observed among the counties and farms within counties could be due to factors such as types of tomato variety cultivated, cropping system and practices; together with the pest management options adopted by the farmers. Kwale County consistently had the highest disease prevalence, incidence presumably due to intense production of sweet potato and cassava crops that are alternative hosts of whiteflies known to be vectors of TYLCV (Díaz-Pendón*et al.,* 2010). However, there is need for further research to establish the role of these host crops in the TYLCD epidemiology.

The presence of TYLCD in all the other counties such as Kirinyaga, Taita Taveta, Meru, Nakuru, Baringo and Kajiado could be linked to the intensive horticultural farming in those counties. Other than tomato, these counties engage in intensive production of crops such as chilli pepper, eggplants, sweet potatoes, beans, and cucurbits; most of which serve as alternative hosts of TYLCD; or harbour whitefly vectors of TYLCV and other begomoviruses.

During the surveys, we observed that farmers practiced crop rotation using non-solanaceous crops. Due to the small land sizes, farmers attempt to avoid the risk of yield loss by growing several crops simultaneously. This created a complex agro-ecosystem that exposes tomato to pests. Moreover, these small land sizes are known to limit long crop rotations (Mwangi *et al.,* 2015). Macharia *et al*. (2015) noted that tomato production within these growing areas surveyed relied on irrigation, thus allowing for continuous cropping throughout the year. This may explain the high prevalence of TYLCD across all counties surveyed; except in Bungoma where tomato production is entirely dependent mainly on rainfall. In a previous investigation by Bob *et al*. (2005), Bungoma had zero prevalence of TYLCD; and Macharia *et al*. (2015) reported no incidence of tomato spotted wilt virus in tomato crops in this County. The low disease prevalence of TYLCD in Bungoma could be attributed to the fact that tomato crop is a secondary crop after maize and it is grown only during the short rainy season.

TYLCD among the most cultivated tomato varieties. This study established that the highest disease incidence was found within the recycled seed (Table 2). The use of recycled seed is considered a cheaper option of obtaining seeds as there in no upfront cost for the farmer, thus making it a common practice amongst resource limited farmers (Japhether *et al*., 2006). Through breeding, TYLCD resistant varieties have been developed and are available in Kenya, although their cost may be prohibitive to majority of small scale farmers. Ochilo *et al.* (2019) observed that varietal characteristics, cost of seed and use influenced the choice of tomato varieties grown in Kenya. It is widely known that most farmers easily opt for cheaper seeds, and varieties with good processing and market qualities like longer storage life (Ochilo *et al.,* 2019). This is contrary to findings in Karnataka, Southern India where majority of farmers (51%) used hybrid tomato seed, mainly due to their high yielding ability and disease resistant (Nagaraju

et al., 2002). Conversely, some of the varieties bred for TYLCD resistance were found to be infected by the virus. Therefore there is need to test tomato varieties under TYLCD pressure to assess their levels of resistant or susceptibility. Resistance to TYLCV in tomato may be broken as a result of rise in virulent TYLCV strains due to factors such as mutations, recombination, inclusions of satellites and the invasion of alien whiteflies (Hosseinzadeh *et al*., 2014; Yan *et al*., 2018). Therefore, it is important that breeders adopt strain specific resistance breeding programmes as this would be more beneficial in tomato breeding.

Identification of virus associated with TYLCD. The use of TYLCV specific antibodies for ELISA in this study was able to successfully confirm the occurrence of TYLCD within the samples (Table 1). However, other viruses could be present within the plant as ToLCArV, ToLCUV and CpCDV have all been reported to be associated with leaf curl on tomatoes in Kenya (Avedi *et al.,* 2020; Kimathi *et al*., 2020; Avedi *et al.,* 2021). Serological assays targeting these viruses could offer more insights into the possible occurrence of mixed virus infections as opposed to singular TYLCV detection.

CONCLUSION

This study has confirmed TYLCD is present in major tomato growing counties in Kenya. The disease incidence and severity varied across the counties based on factors such as types of tomato variety cultivated, cropping system and practices; together with the pest management options adopted by the farmers. Kwale and Baringo Counties had the highest disease incidence and severity, respectively; while Bungoma had the least. Recycled seed had highest disease incidences and severity; while hybrids had the least. Extensive studies should be conducted to establish the diversity of the casual agents of TYLCD in Kenya in both cultivated and uncultivated host plants. This will provide useful information towards breeding for virus and strain specific resistance together with the adoption of technologies like gene pyramiding in order to develop varieties with durable resistance. To ensure that TYLCD-resistant varieties are adopted by farmers, farmer-preferred traits should be incorporated during breeding. There is also the need to sensitise farmers on the importance of using certified seeds.

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