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SEASONAL DYNAMICS AND ALTERNATE HOSTS OF THRIPS TRANSMITTED *Iris yellow spot virus* IN KENYA

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ABSTRACT

Thrips-transmitted *Iris yellow spot virus* (IYSV) (Family *Tospoviridae*, Genus *Orthotospovirus*) is a major constraint to onion (*Allium cepa* L.) production in Kenya. Determining seasonal patterns of the vector and alternate hosts of the virus could help onion farmers plan Integrated Pest Management strategies; while allowing them to move away from calendar-based applications of insecticides. The objective of this study was to determine the distribution, seasonal variations and alternate hosts of vector and IYSV. For distribution, a survey was carried out on a network of farms in all onion growing areas in Kenya; while for seasonality, surveys were done in two areas; Loitoktok and Naivasha. Data were collected on IYSV incidence, thrips population and alternate hosts. Results showed IYSV was widely distributed in all onion growing areas; with incidence varying from 26 to 72%. Highest IYSV incidence was recorded during the cool-dry season, and varied from 56.5 to 71%; while lowest IYSV incidence in onions was observed during the cool and wet season (29.9 to 32.2%). Iris yellow spot disease incidence positively correlated with the number of onion thrips in Loitokitok ($r = 0.659$; $P<0.0001$) and Naivasha ($r = 0.623$; $P<0.0001$). Identified alternate hosts for IYSV were leeks, chives, shallots, lambsquarters, redroot pigweed, Chinese lantern and black nightshade.Occurrence of thrips on onions, which is grown all year round calls for urgent integrated pest management strategies that includes host plant resistance, field sanitation, forecasting and use of beneficial insects and parasitoids to reduce impact of the pest and disease. Plant health adherence through removal of alternate weeds hosts around the cultivated fields, would be useful in minimising IYSV incidence.

Key Words: *Allium cepa*, chives, lambsquarters, leeks, shallots, tospoviruses

RÉSUMÉ

Le virus Iris de la tache jaune (IYSV) transmis par les thrips (Famille *Tospoviridae*, Genre *Orthotospovirus*) est une contrainte majeure de la production de l'oignon (*Allium cepa* L) au Kenya. Déterminer la distribution saisonnière du vecteur et les hôtes alternatives des virus pourrait aider les producteurs d'oignon à mettre en place des stratégies de gestion intégrée des pestes, en vue de leur permettre d'éviter les applications des insecticides sur la base du calendrier. L'objectif de cette étude était de déterminer la distribution, les variations saisonnières et les hôtes alternatives du vecteur et de IYSV. Pour la distribution, une enquête a été conduite sur un réseau de champs dans toutes les zones productrices d'oignon du Kenya ; alors que pour la saisonnalité, des enquêtes ont été conduites dans deux zones ; Loitoktok et Naivasha. Les données ont été collectées sur l'incidence d'IYSV, la population de thrips et les hôtes alternatives. Les résultats ont montré que IYSV était largement distribué dans toutes les zones de production d'oignon ; avec l'incidence variant de 26 à 72%. La plus grande incidence d'IYSV a été obtenue pendant la saison sèche et froide, et varie de 56,5 à 71% alors que la plus faible incidence de l'IYSV

sur les oignons a été observée pendant la saison humide et froide (29,9 à 32,2%). L'incidence de la maladie de la tache jaune de Iris était positivement corrélée avec le nombre de thrips des oignons à Loitokitok (r = 0,659; P<0,0001) et Naivasha (r = 0,623; P<0,0001). Les hôtes alternatives identifiées pour l'IYSV étaient des poireaux, les ciboulettes, les échalotes, les ambsquarters, les amarantes à racine rouge, la lanterne chinoise et la morelle noire. L'occurrence des thrips des oignons produits toute l'année appelle à des stratégies urgentes de gestion intégrée des pestes qui comprennent la résistance de la plante hôte, l'assainissement du champ, prévision, et l'usage des insectes bénéfiques et parasitoïdes pour réduire l'impact de la peste et de la maladie. Le respect de la santé de la plante à travers l'enlèvement des adventices, les hôtes alternatives dans les champs cultivés, serait bénéfique en minimisant l'incidence de l'IYSV.

Mots Clés: *Allium cepa*, chénopode blanc, ciboulette, échalotes, poireaux, tospovirus

INTRODUCTION

Bulb onion (*Allium cepa* L.) is among the most important vegetable crops for domestic markets in Eastern Africa (FAOSTAT, 2010). However, its production does not meet the local demand as a result of losses resulting from pests and diseases. Onion thrips (*Thrips tabaci* Lindeman; *Thysanoptera: Thripidae*) is a polyphagous pest that causes serious damage on bulb onions all over the world (Pappu *et al.,* 2009; Bag *et al.,* 2015). Larvae and adult thrips cause direct damage, by destroying epidermal cells through piercing surface tissues and sucking exudate cellular contents (Koschier *et al*., 2002). The damaged cells on attacked plants create silvery-white patches that reduce the photosynthetic area, resulting in low yield and susceptibility of the plant to opportunistic pathogens (Gent *et al.,* 2006; Pappu *et al.,* 2009). Onion thrips also cause indirect damage to the onions, by transmitting Iris yellow spot virus, an important plant pathogen worldwide (Bag *et al.,* 2015). Individual vector thrips acquire the virus as first instar and remain viruliferous for life as the virus replicates in propagative manner within insect's tissue (Nagata *et al.,* 2004; Srinivasan *et al.*, 2012)

Onion growers in Kenya use insecticides to control onion thrips; while fungicides are incorrectly applied to control IYSV, which has been misidentified as the fungal disease purple blotch. These pesticides are sprayed throughout the cropping season, with up to 12 to 15 spray times (Waiganjo *et al.,* 2006).

Conventional insecticides are not only ineffective in controlling thrips due to insecticide resistance development, but are also harmful to the environment (Waiganjo *et al.,* 2008). In order to have an effective integrated disease management strategy, information on seasonal variations and alternate hosts of IYSV is a pre-requisite. Knowledge on seasonal pest dynamics allows farmers to deploy pesticide sprays and other control tactics effectively, thus increasing their value and decreasing negative impacts on the environment (Nault and Shelton, 2010). Moreover, when integrated pest management tactics are timed properly with key pest development stages, they can minimise the need for subsequent insecticide treatments (Wilby and Thomas, 2002; Herms, 2004). Alternate hosts for the IYSV serve as a bridge allowing the virus to survive between onion-growing seasons.

Although alternate hosts for IYSV have been detected in onion related crops and weed species in many parts of the world (Cosmi *et al.*, 2003; Sampangi and Mohan, 2007; Hsu *et al.,* 2011), no such research has been done in Kenya. Hence, the purpose of this study was to determine the distribution, seasonal abundance and alternate hosts of onion thrips and IYSV in onion growing areas in Kenya.

MATERIALS AND METHODS

Study sites and sample collection. A survey was conducted between March and December 2012, to assess the distribution of thrips and IYSV in onion growing areas

of Subukia, Embu, Mwea, Thika, Makueni and Loitokitok. In each region, seven onion farms were surveyed and in each farm 10 plants were sampled at random and visually checked for presence of IYSV infection that is characterised by chlorotic or necrotic, white to straw-coloured dry, elongated or spindle diamond-shaped lesions along the scape. Testing of IYSV infection in the field was done using Agdia IYSV lateral flow immunostrips (Agdia, Elkhart, IN) and further confirmed in the laboratory using double antibody sandwich Enzyme linked Immunosorbent Assays (DAS-ELISA) as described by Pappu *et al.* (2009).

Monthly surveys were conducted in Loitokitok (2.73 °S: 37.51 °E) and Naivasha (0.48 °S: 37.26 °E) for three seasons: long rains season (March to May), cool and dry season (July to September) and short rains season (October to December). The study sites lies between 1200 and 1800 m above the sea level and receives bimodal rainfall of between 677 and 894 mm per annum. The mean temperatures range from 17.1 to 17.8 °C and the areas comprise of clay to sandy soils.

Determination of IYSV incidence, severity and thrips population. Disease incidence was determined as the percentage of infected bulb onion plants in four randomly selected 1 m^2 grids. Disease severity was scored as the percentage of the leaf surface showing IYSV symptoms, on a scale of 0 to 5; where 0 = no symptoms, 1 = 1 to 25% of foliage diseased, 2 = 26 to 50% of foliage diseased, 3 = 51 to 75% of foliage diseased, 4 = 76 to 80% of foliage diseased and 5 = 81 to 100% of foliage diseased (Clinton *et al.,* 2008). Thrips population levels were assessed on ten randomly selected plants per farm, by tapping each plant over a white enamel tray. Thrips were then collected and preserved in 95% alcohol in a 1.5 ml Eppendorf tubes, later identified after processing and mounting on slides as outlined in the LuCID key "Pest thrips of the world" (Moritz *et al.,* 2001).

Identification of IYSV in alternate hosts plants. Several weeds within and around the onion farms were collected and tested for IYSV. They included blue couch grass (*Digitaria abyssinica),* bristly foxtail (*Setaria verticullata)*, goat weed (*Ageratum conyzoides*), black jack (*Bidens pilosa),* double thorn (*Oxygonium sinuatum*), common purslane (*Portulaca oleracea),* pig weed (*Amaranthus retroflexus*), lambsquarters (*Chenopodium album*), Chinese lantern (*Physalis minima)*, Jimson weed (*Datura stramonium*), Marigold (*Tagetes minuta)* and black nightshade (*Solanum nigrum*). Shallots and leek crops species showing IYSV infection were also sampled at random, and symptomatic portions of the samples tested for presence of the virus using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR).

Extraction of RNA and Complementary DNA (cDNA) synthesis. About 100 mg of the leaf samples obtained from suspected IYSV infected alternate hosts were ground in liquid nitrogen, and total RNA extraction was done using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA), following manufacturer's protocol. Purified RNA was stored at -80 °C in RNase free water. Reverse transcription was carried on the extracted RNA using Herc II reverse transcriptase (Strategene), to generate complimentary DNA (cDNA), as described by Pappu *et al.* (2009) with slight modifications. To a nuclease-free 1.5 ml micro-centrifuge tube, the following three components were put in the order of 2 µl of total RNA, 3 µl random primers and 9.2 µl of RNase-free water. The mixture of RNA template, water and primers was incubated at 65 °C for 5 minutes, and slowly allowed to cool to 25 °C, and then incubated for 10 minutes to allow primers to anneal to RNA. The following four components were then added to make the final reaction volume to 20 µl; 2 µl 10× Affinity Script RT buffer, 2 µl 100 mM DTT, 0.8 µl 100 mM dNTPs mix

and 1 µl of Affinity Script multiple temperature, Reverse Transcriptase (RT). The RNA-reverse transcriptase mixture was then incubated at 25 °C for 10 minutes; followed by temperature raise to 42 °C for 1 hour to allow complementary DNA synthesis that was used for subsequent steps.

PCR amplification, purification and sequencing. The synthesized complementary DNA was used as the template for PCR amplification of the IYSV specific nucleocapsid gene. After incubation of RT reaction, 25 µl of the PCR mix containing 12.5 µl of hot start Taq polymerase master mix (Qiagen), 0.5 µl 20 mM forward primer IYSV-465c: 52 -AGCAAAGTGAGAGGACCACC-32 and 0.5 µl 20 mM reverse primer IYSV-239f: 52 -TGAGCCCCAATCAAGACG-32 and 9.5 µl of RNase free water was added to the 2 µl of the cDNA. The mixture was then subjected to thermal cycling, with initial denaturation of 94 °C for 15 min and 35 cycles of (30 sec denaturation at 94°C, 30 sec annealing at 52 °C and 30 sec extension at 72 °C). The final extension at 72 °C was incubated for 5 min using PTC-100™ thermo cycler (Research Inc.).

Amplified PCR products were analysed on a 1% agarose gel stained with 3 µl (0.5 µg ml^{-1}) ethidium bromide in 1× TAE buffer at 70V for 1.5 hours. The gel was then visualised under a UV trans-illuminator. The amplicons of expected sizes of approximately 200 bp were scored on the gel and purified using Quick Clean II gel Purification kit (GenScript, UK), following manufacturer's instructions. The purified PCR products were sequenced using respective IYSV forward and reverse primers. Sequencing of the PCR product was performed by the dideoxynucleotide chain termination method (Sangler *et al.,* 1977) at Macrogen, Seoul, Korea. Consensus sequence was generated using Geneious Pro 5.5.6 Software (Bio matters Ltd., Auckland, NZ).

Data analysis. Data on thrips population dynamics, IYSV incidence, severity and virus titre level were subjected to Analysis of Variance (ANOVA) and multiple comparisons of means at 95% significance was carried out using Student Newman-Keuls (SNK) test. Pearson correlation analysis was performed to determine the relationship between thrips number and IYSV incidence. All statistical analyses were conducted using R version 2.10 (R Development Core Team, 2009) using packages "Rcmdr version 1.5-3" (Fox *et al.,* 2009). Basic Local Alignment Search Tool (BLAST) was performed to compare the sequences with IYSV sequences already available in the GenBank database. Multiple alignments of the sequences were done using Clustalw.

RESULTS

Iris yellow spot disease incidence, severity and titre levels. Iris yellow spot disease incidence varied significantly among the areas surveyed ($F_{5, 72} = 105.08$; $P<0.0001$) with highest incidence observed in Mwea and Loitokitok (72%); while the least incidence was recorded in Subukia (26%) (Fig. 1).

Iris yellow spot disease was detected in all the locations surveyed. It was observed that IYSV infected leaves had an island of green tissue that developed in the centre of the necrotic area (Fig. 2A). As more lesions developed and increased in size, they coalesced, often completely girdling and reducing the photosynthetic area of the leaf scape. Depending on the age of the onion crop, infection was found to be associated with less defined symptoms on the leaves that were confused by growers with those caused by downy mildew or purple blotch disease (Fig. 2B-D). Additionally, it was observed that the necrotic areas resulting from IYSV infection were colonised by secondary infections that contributed to early necrosis of tissues (Fig. 2B-D).

There was significant difference in the disease severity among the onion growing areas, varying from 1.58 to 3.96 (Table 1). The highest severity was observed in

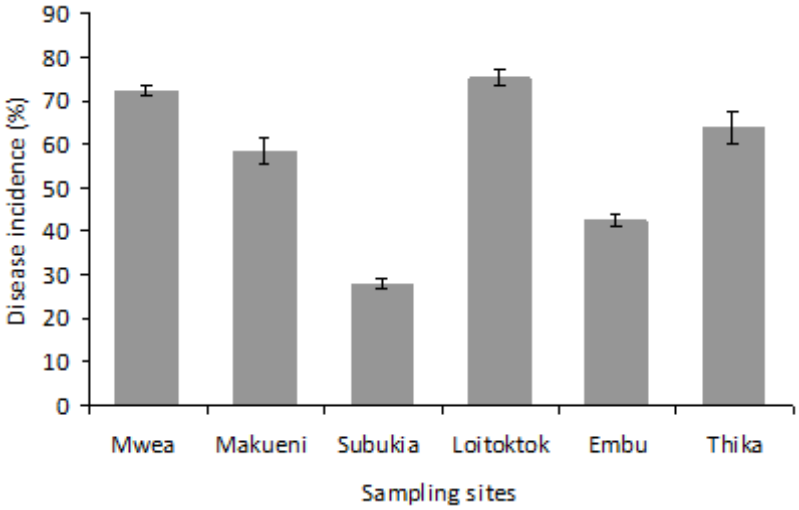

Figure 1. Incidence of Iris yellow spot virus in the six onion growing regions. Bars represent the standard errors of the means of IYSV incidence recorded in seven farms surveyed.

Figure 2. Symptoms of *Iris yellow spot virus* : Where (A) and (B) Spindle-shaped straw coloured chlorotic lesions with occasional green islands on leaves; (C) drying of leaves due to coalescing of individual lesions and secondary infection leading to necrosis and (D) IYSV infected onion field showing extensive drying of leaves.

TABLE 1. Iris yellow spot disease severity and virus titre levels at 405 nm onion obtained from selected onion growing regions in Kenya

Location	Altitude (m)	Disease severity	Virus titre levels at 405 nm
Loitokitok	1200	3.96±0.09a	3.31±0.18a
Mwea	1200	3.45±0.08b	2.41±0.14b
Makueni	1219	2.78±0.08c	2.81±0.12ab
Embu	1480	2.61±0.08c	1.09±0.08c
Thika	1631	3.69±0.16ab	2.95±0.36 ab
Subukia	1950	1.58±0.11d	1.02±0.08c
F-value		$F_{5, 442} = 50.26$	$F_{5, 149} = 45.10$
P-value		$P < 0.0002$	$P < 0.0002$

Within a column, means followed by different letters ($P < 0.05$) are significantly different Student Newman's Kleus (SNK) test. Values are mean disease severity and virus titre per location ±S.E

Loitokitok, followed by Thika, Mwea, Makueni and Embu. Subukia had the least disease severity score. The virus was not present in non-symptomatic host tissue and was only detected by DAS-ELISA in tissue sampled within 3 to 5 cm of visible lesions. The mean optical density of the virus load was highest in Loitokitok, and lowest in Subukia (Table 1). Iris yellow spot disease severity positively correlated with virus load ($r = 0.793$; $P < 0.0001$)

Thrips species composition. Onion crop in the six areas surveyed was infested by more than one thrips species. Onion thrips (*Thrips tabaci* Lindeman) was the dominant species, constituting 81.3% of the total insects collected; followed by Western flower thrips; *Frankliniella occidentalis* (Pergande) (6.54%), cotton bud thrips; *F. schultzei* (Trybom) (3.63%), bean flower thrips; *Megalurothrips sjostedti* (Trybom) (1.42%), *Scirtothrips dorsalis* Hood (1.61%), *Hydatothrips* sp. 0.64% and Tubuliferan thrips 1.92%. Thrips predator, *Orius,* constituted 0.56% of the total insects collected; while parasitoid *Ceranius menes* (Walker, 1839) represented 2.39% of the total collections. *Frankliniella occidentalis* was frequently observed in onion farms in Mwea, where it accounted for 15.1% of the total insects infesting onions in that area.

Thrips seasonal variations. Significant variation in number of thrips was found over the sampling times in both regions; Loitokitok ($F_{8, 54} = 51.393$; $P<0.0001$) and Naivasha ($F_{8, 54} = 25.396$; $P<0.0001$) (Table 2). In Loitoktok, the highest numbers of thrips was observed in August; while in Naivasha it was recorded in September, which coincided with cool and dry weather season in both regions. The lowest thrips numbers in both regions was recorded from October to December that coincided with short rain season (Table 2). Thrips numbers positively correlated with IYSV incidence in Loitokitok ($r = 0.659$) and Naivasha ($r = 0.623$).

Seasonal variation in IYSV incidence and severity. The incidence of IYSV varied significantly over sampling times in Loitokitok ($F_{8, 54} = 88.176$; $P<0.0001$) and Naivasha ($F_{8, 54} = 81.696$; $P<0.0001$), with the highest IYSV incidence recorded in the cool and dry season in August through September (Table 3). Severity of IYSV also varied over sampling times, and the highest was recorded in Naivasha and Loitoktok in the months of August and September (Table 3). The lowest disease incidence and severity in the two locations were recorded in short rain season (October to December). It was also observed that 95% of the farmers in Loitoktok grew

TABLE 2. Number of onion thrips per month in Loitokitok and Naivasha over three cropping seasons in Kenya

Season	Sampling months	Loitokitok	Naivasha
Long rain	March	22.1 ± 0.46b,c	13.47 ± 0.47d
	April	24.98 ± 0.77b	17.57 ± 0.68b,c
	May	21.67 ± 0.52bc	12.03 ± 1.02d
Cool and dry	July	25.57 ± 0.40b	16.2 ± 1.02c
	August	30.04 ± 1.64a	19.49 ± 1.07a,b
	September	25.56 ± 1.92b	20.96 ± 0.36a
Short rain	October	18.96 ± 0.60c	12.92 ± 0.89d
	November	7.67 ± 1.19d	11.93 ± 0.38d
	December	7.94 ± 1.16d	8.14 ± 1.01e
$F_{8, 54}$		51.393	25.396
P-value		P < 0.0001	P < 0.0001

Within a column, means followed by different letters are significantly different (P<0.05) using Student Newman Kleus (SNK test). Values are mean adults onion thrips per plant ± S.E

TABLE 3. Incidence and severity of IYSV over the sampling time in the three cropping seasons in Loitokitok and Naivasha

Seasons	Sampling time	Incidence		Severity	
		Loitokitok	Naivasha	Loitokitok	Naivasha
Long rain	March	48.38 ± 1.56d	33.08 ± 1.44e	1.98 ± 0.04c	1.83 ± 0.06a
	April	53.57 ± 1.35c	24.43 ± 1.75d	2.19 ± 0.06bc	1.84 ± 0.04a
	May	57.31 ± 1.37bc	51.70 ± 1.04	2.21 ± 0.09bc	1.87 ± 0.05a
Cool and dry	July	60.54 ± 1.35b	53.23 ± 1.10bc	2.34 ± 0.09b	1.89 ± 0.15a
	August	69.89 ± 1.87a	56.46 ± 0.90ab	2.98 ± 0.08a	1.93 ± 0.06a
	September	70.66 ± 2.34a	60.2 ± 1.12a	2.87 ± 0.12a	1.93 ± 0.06a
Short rain	October	37.18 ± 2.40e	28.74 ± 1.56e	1.34 ± 0.12d	1.23 ± 0.02b
	November	38.01 ± 1.29e	31.97 ± 1.47e	1.33 ± 0.07d	1.23 ± 0.04b
	December	40.78 ± 1.16e	32.23±1.52e	1.31 ± 0.06d	1.27 ± 0.07b
$F_{8,54}$		88.176	81.696	51.244	39.918
P-value		P < 0.0001	P < 0.0001	P < 0.0001	P < 0.0001

Within a column, means followed by different letters are significantly different (P<0.05) using Student Newman Kleus (SNK test).

Red creole variety; while in Naivasha100% of farmers grew Texas grano variety.

Alternate hosts for *Thrips tabaci* and IYSV. Cultivated crops that were infested with high numbers of onion thrips were cabbage (32.2±5.2), leeks (28.2±3.9), chives (25.4±4.5) green bunching onions (24.1±5.7), shallots (36.9±3.1), snap peas (21.1±5.1) and potatoes (18.8±4.5). Among these crops, only leeks, chives and shallots produced symptoms that were characteristic of IYSV infestation.

TABLE 4. Nucleocapsid gene percentage level of similarity for the identified IYSV alternate hosts in Kenya and isolates deposited in the Genbank

Alternate host(s)	Level of similarity (%)	Isolate source	Gene bank accession no.
Lambsquarters	94	Sri Lanka	GU901211
	93	Iran	HQ148173
Jimson weed	94	Iran	HQ148173 and
	94	Australia	KJ769192
Redroot pigweed	97	Kenya	HQ711616.1
	95	India	EU310278.1
Chinese lantern & black nightshade	97	Kenya	HQ711616.1
	95	Sri Lanka	GU901211
Chives and shallots	98	Kenya	HQ711616.1
	97	Iran	HQ148173.1
Leeks	96	Kenya	HQ711616.1
	95	Sri Lanka	GU901211
	94	India	KF624624

Weed species that were alternate hosts for IYSV infection were redroot pigweed (*Amaranthus retroflexus*), lambsquarters (*Chenopodium album*), Chinese lantern (*Physalis minima)*, Jimson weed (*Datura stramonium*) and black nightshade (*Solanum nigrum*), although they did not produce any distinct characteristic for IYSV infection.

Amplicons of approximately 200 bp were obtained from IYSV positive samples targeting nucleocapsid gene. The IYSV positive lambsquarters isolate had the highest nucleotide sequence identity with the corresponding IYSV isolates from Sri Lanka, followed by the isolates from Iran. Jimson weed IYSV isolate samples showed the highest nucleotide sequence identity with the corresponding IYSV isolates from Iran and Australia (Table 4). Redroot pigweed IYSV isolate showed the highest nucleotide sequence identity of 97%, with the corresponding region of IYSV isolates obtained from Kenya followed by 95% sequence identity with isolates from India.

Chinese lantern and black nightshade had highest nucleotide identity of 97% with IYSV isolates obtained from bulb onions in Kenya; followed by IYSV isolates from Sri Lanka (Table 4). Chives and shallots IYSV positive isolates showed the highest nucleotide sequence identity of 98%, with IYSV isolates obtained from bulb onions from Kenya; followed by isolates from Iran (Table 4). Leeks showed highest nucleotide identity of 96% with IYSV isolates obtained from bulb onions from Kenya; followed 95% similarity with IYSV isolates obtained from Sri Lanka and 94% nucleotide similarity with IYSV obtained from India (Table 4).

DISCUSSION

Thrips species composition and Iris yellow spot disease incidence. Onion thrips were abundant in all the onion growing areas and contributed to high IYSV incidence (Fig. 1). This is attributed to the fact that iris yellow spot disease is transmitted by adult onion thrips in a persistent propagative manner, after acquisition of the virus by larvae thrips. This is in agreement with Kritzman *et al*. (2001),

who observed high population of *T. tabaci* on onions in Israel and attributed them to IYSV incidence. Mid-altitude areas of Mwea, Thika and Loitokitok of Kenya had high IYSV incidence compared to the high altitude regions of Subukia. This could be due to difference in temperature and humidity experienced in these regions (Jaetzold and Schmidt, 1983). In mid-altitude regions (Mwea, Thika and Loitokitok), mean temperature ranges from 25-30 ºC, with a mean annual rainfall range of 200-700 mm (Jaetzold and Schmidt, 1983), which favour higher rate of T. tabaci development and reproduction (Waiganjo *et al.,* 2008); and subsequent high virus spread. Subukia lies in the high altitude region and experiences high humidity, with annual rainfall ranging from 1100-1200 mm with moderately low temperature (21-24 ºC) (Jaetzold and Schmidt, 1983), which lowers the rate of *T. tabaci* reproduction, and hence low virus spread. Similar observations were made by Waiganjo *et al.* (2008), who reported that dry weather, with moderately high temperatures, increased seasonal thrips numbers; while wet seasons with moderately high relative humidity negatively correlated with thrips number.

Seasonal variation in onion thrips and IYSV incidence. Although onion thrips population density varied in both Loitoktok and Naivasha, seasonal trend was similar at all sampling times (Table 2). Thrips population density was low in the wet season (October to December), but high in the cool and dry season (July to September). High humidity experienced during wet season lowered thrips reproduction and their dispersal rate; while increasing their developmental period. These findings concur with Shannon *et al.* (2008), who reported that high temperature and precipitation affected seasonal patterns of tobacco and onion thrips. Lorini and Junior (1990) reported that lack of rainfall increased thrips population density of T. tabaci on garlic in Brazil. Hamdy and Salem (1994) reported that female onion thrips laid most eggs and lived longest within temperatures of 21.1 to 23.6 oC and relative humidity of 52%. Heavy rains have been reported to wash thrips off plants down to the soil surface, causing sharp declines in their population density (North and Shelton, 1986).

Seasonality survey showed significant variation in IYSV incidence in Loitoktok and Naivasha (Table 3). Loitokitok recorded the highest IYSV incidence and severity in all the sampling times compared to Naivasha. Differences in the varieties grown by farmers could have played a key role, although other factors such as field sanitation, warm environment and high thrips population may not be excluded. Red creole was widely grown in Loitokitok, which is susceptible variety to onion thrips and IYSV; while Texas grano a moderately resistance variety to onion thrips and IYSV was commonly grown in Naivasha (Kibayu, 2009; Birithia *et al*., 2014). DiazMontano *et al*. (2010) also reported differences in 46 onion germplasms to onion thrips and IYSV. In addition, Loitoktok lies in medium altitude area of 1200 msl; while Naivasha lies in high altitude area of 1800 msl that is characterised by low temperatures that lower the reproduction rate of onion thrips.

Alternate hosts for Thrips tabaci and IYSV. Lambsquarters, redroot pigweed, Chinese lantern and black nightshade were abundant in onion growing areas and acted as IYSV alternate hosts (Table 4). Evans et al. (2009) reported that green fox tail, a naturally occurring weed, was a reservoir for IYSV. Similar observations were made by Smith et al. (2011), on onions in New York who reported four weed species including Arctium lappa (common burdock), Taraxacum officinale (dandelion), Rumex crispus (curly dock) and Cichorium intybus (chicory) as a reservoir of IYSV. Similarly, Szostek and Schwartz (2015) reported dandelion, flixweed, prickly lettuce and salsify as a direct host of Iris yellow spot virus.

CONCLUSION

The disease incidence was recorded in cool and dry season (July-September) in both Loitoktok and Naivasha; and positively correlated with the number of onion thrips during the growing seasons. This implies that onion thrips presence plays a major role in the transmission of IYSV. The continuous occurrence of thrips on onion field's in all seasons call for immediate integrated pest management strategies to combat the menace. Plant health adherence through removal IYSV alternate host crops and weeds around the cultivated fields would be useful in minimising impact of the disease.

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