DETECTION OF WEED SPECIES INFECTED BY *Tomato ringspot virus* IN FIELD-GROWN TOMATO IN SUDAN SAVANNA, NIGERIA.

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SUMMARY

Field study was conducted to determine the occurrence and distribution of weed hosts of Tomato ringspot virus (ToRSV) in field-grown tomato in Gombe, Jigawa and Kano States in the Sudan savanna regions of Nigeria during the 2017 and 2018 dry and wet seasons. Three farms each from three Local Government Areas (LGAs) of each state were surveyed. Ten symptomatic and asymptomatic weed samples within and around each farm (n=1080) were randomly collected and assayed against ToRSV using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). The results showed that 19 weeds species from 11 families were established as alternative host of ToRSV in all States surveyed but with variation in distribution. Kano States had significantly ($p \leq 0.05$) higher number (12) of weed hosts (12) of ToRSV compared to five weed species each in Gombe and Jigawa States. Solanum nigrum L. significantly ($p \leq 0.05$) had the highest frequency rating (10.3 %) of ToRSV. From available literature, this is the first report of ToRSV naturally infecting the 19 weed species in Nigeria. The findings of the present study point to a better understanding of ToRSV disease epidemiology for its effective management. It is recommended that similar studies be carried in others adjoining states to identify potential weed hosts of ToRSV. Molecular characterization of the virus isolates of the virus should be conducted for the possible existence of new strain(s) in the region.

Keywords: Alternative weed host, detection, distribution, ToRSV

Tomato ringspot virus (ToRSV) is considered one of the most damaging members in the genus of the family *Secoviridae* (CABI, 2018). The particles of the virus are unstable, icosahedral in shape with tripartite components and having a single-stranded RNA of 28 nm in diameter (Samuitiené *et al.,* 2003). Two components of the positive sense ssRNA (RNA1 and RNA2) share the infectious genome of ToRSV with 5' and 3' ends of each having a VPg and being polyadenylated (Stace-Smith, 1996; Sanfaçon, 2008; Sanfaçon *et al.,* 2009). About 285 plant species in 159 genera belonging to 55 families are reported to be infected by ToRSV (Edwardson and Christie, 1997; EPPO, 2018), causing diseases of economic importance in perennial woody and cultivated annual plants in nature (Šneideris *et al.,* 2012). These include cucumber, tomato, raspberries, grapes, peaches, cherries and other *Prunus* spp., black currants, gooseberries, strawberries, Pelargonium, Hydrangea, *Fraxinus americana* and Gladiolus (OEPPO/EPPO, 2001; EPPO, 2005; Zitikaité and Staniulis, 2006; Tzanetakis and Martin, 2013; González *et al.,* 2017). Mottling of leaves with circular chlorotic spots and ringspots of similar size on leaves and fruits and stunted growth of infected plants are common symptoms induced by ToRSV (Fuchs *et al.,* 2010; Tuffen, 2018). ToRSV transmission readily occurs through vegetative propagation, sap inoculation, seeds and pollen (EPPO, 2005; CABI, 2018). However, species of *Xiphinema* and *Longidorus* remain the most important transmitting agents of the virus in many susceptible host plants (Tomlinson, 2014). Infected seeds may serve as a source of primary inoculum of virus in the soil (Sastry, 2013). ToRSV causes significant economic yield losses in many perennial fruit crops, ornamentals and horticultural crops worldwide (Griesbach, 1995; Stace-Smith, 1996; Basso *et al.,* 2016). The virus has been reported to be widely distributed in northern Nigeria, infecting field grown tomato crops with varying incidences (Abraham *et al.,* 2019b).

The management of ToRSV is further complicated by the prevalence of weed species serving as reservoir or alternative hosts of both the virus and its vectors in nature. Moreover, seeds of some weed species have also been implicated for the successful transmission of the virus (Sastry, 2013). Although ToRSV has been reported to be widespread infecting both cultivated and uncultivated plant hosts (EPPO, 2018), there is paucity of information on the natural occurrence of the virus on alternative weed hosts in tomato fields in northern Nigeria where the bulk of tomato production in the country lies (GEMSA4, 2016). Detection of alternative weed hosts of ToRSV is an important step towards curbing the disease spread and to ensure effective management measures (Martinelli *et al.,* 2015). Therefore, the present study reports weed species infected by ToRSV in field-grown tomato fields in Northern Nigeria.

MATERIALS AND METHODS

Survey and Sampling locations

Studies on the weed hosts of ToRSV were carried out during the 2017 and 2018 dry and rainy seasons in three tomato fields in each of three Local Government areas (LGAs) in three major tomato cultivating States (Gombe State: Akko, Kaltungo and Yamaltu-Deba LGAs; Jigawa State: Hadejia, Kirikasama and Kazaure LGAs and Kano State: Garun Mallam, Bagwai and Kura LGAs) in Northern Nigeria.

Weed sampling and identification

Ten symptomatic and asymptomatic weed samples from each tomato field (n=1080) were randomly collected within and around one metre (1m) of tomato fields. Each sample was placed in a polyethylene bag, labelled and kept on an ice chest. The weeds were identified at the Herbarium of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University, Zaria and according to their description by Akobundu *et al.* (2016). Prior to further investigation, samples were stored at 4°C at the Virology Unit, Department of Crop Protection, Ahmadu Bello University, Zaria.

Serological assay

Weed samples were indexed against ToRSV using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using specific antisera supplied by Leibniz-Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen Gmbh, Braunschweig, Germany. The antigen–antibody reactions were detected and optical density (OD) were recorded after 1 h using an ELISA plate reader Uniequip (Martinseed, Germany) at 405 nm (Clark and Adams, 1977). Positive (ToRSV infected tomato leaf sample obtained from DSMZ, Germany) and negative controls (Healthy tomato leaves) were included in each plate. Values at least twice that of the negative control were considered positives (Kumar, 2009). Samples from weed species that tested positive against ToRSV in both dry and wet seasons were considered as host of the virus.

Data analysis

Data obtained on the incidence of ToRSV in weed species were subjected to analysis of variance. Variation of means were considered significant at 5% level of probability using either least significant difference (LSD) or by plotting standard error of means as described by Gomez and Gomez (1984).

RESULTS

The results in Tables 1-3 present the weeds species assayed against ToRSV in Gombe, Jigawa and Kano States respectively. The results obtained showed that Kano State had significantly ($p \leq 0.05$) higher number (12) of ToRSV weed hosts (*Acanthospermum hispidum* DC., *Ageratum conyzoides* L., *Ipomea eriocarpa* R. Br., *Bidens pilosa* Linn., *Gomphrena celosioides* Mart., *Nelsonia canescen* (Lam.) Sprengs, *Solanum nigrum* L., *Portulaca oleraceae* L., *Chenopodium album* L., *Amaranthus spinosus* L., *Laggeria aurita* (L. f.) DC and *Cassia obtusifolia* L.) while seven weed species each were detected to be infected by ToRSV in Gombe State (*Cassia occidentalis* Linn., *C. obtusifolia* L. *Physalis angulata* L., *Mitracarpus villosus* (Sw.) DC., *Boerhavia diffusa* L., *Corchorus trilocularis* L., and *P. oleraceae*) and Jigawa State (*Lactuca serriola* L., *A. spinosus* L., *S. nigrum* L., *C. obtusifolia* L., *Commelina errecta*, *P. oleraceae* and *P. angulata*) (Fig. 1 and Tables 1-3). *S. nigrum* L. had the highest frequency rating (10.3 %) of weeds infected by ToRSV followed by *C. obtusifolia* (9 %) while *Lactuca serriola* L., *Ipomea eriocarpa* R. Br., *Mitracarpus villosus* (Sw.) DC., *Nelsonia canescen* (Lam.) Sprengs, *Lactuca serriola* L., *Bidens pilosa* Linn., *Chenopodium album* L., *Gomphrena celosioides* Mart., and *Boerhavia diffusa* L., had the lowest frequency of 3.8 % (Fig. 2).

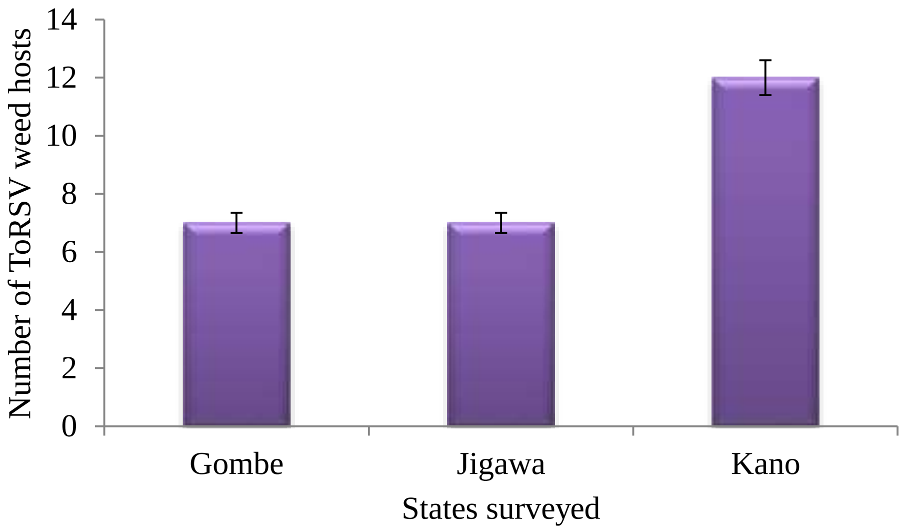

Figure 1: Number of weed species detected as alternative weed hosts of *Tomato ringspot virus* in the Gombe, Jigawa and Kano States. Bars indicate standard error of means at 5% probability level.

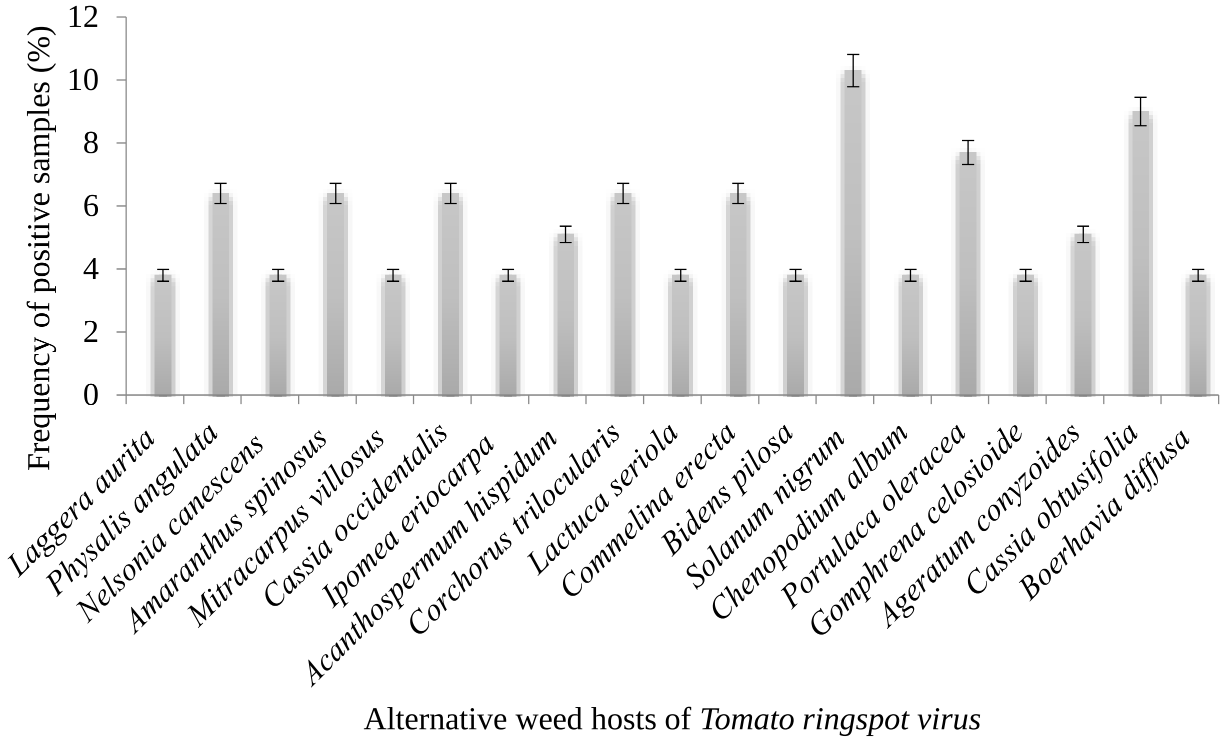

Figure 2: Frequency of positive samples detected in alternative weed hosts of *Tomato ringspot virus*. Bars indicate standard error of means at 5% probability level.

Table 1: Weed species tested against antiserum of *Tomato ringspot virus* in Gombe State during 2017 and 2018 dry and wet seasons.

Weed families/species	2017		2018	
	DS	WS	DS	WS
Asteraceae				
Tridax procumbens	-	-	-	-
Ageratum conyzoides L.	-	-	+	-
Apilia Africana (Pers) D.C.	NF	+	NF	-
Chromolaena odorata (L.) R. M.	-	NF	NF	-
Eclipta prostrata L.	-	-	-	-
Venonia cinerea (Linn) Less	-	NF	-	-
Venonia ambigua	-	-	NF	-
Acanthospermum hispidum DC.	-	-	-	+
Lactuca taraxacifolia (Willd.) Schum	-	-	NF	-
Lactuca serriola	NF	+	-	-
Bidens pilosa Linn.	-	+	-	-
Euphorbiaceae				
Euphorbia hirta L.	-	-	-	+
Phyllanthus amarus Schum	+	-	-	-
Euphorbia hyssopifolia Linn.	-	-	NF	+
Euphorbia heterophylla Linn.	-	-	-	-
Commelinaceae				
Commelina diffusa Burm. f.	-	-	-	-
Commelina benghalensis L	-	-	-	+
Nyctaginaceae				
Boerhavia diffusa L.	+	NF	+	+
Sterculiaceae				
Waltheria indica Linn.	NF	-	-	-
Solanaceae				
Physalis angulata L.	+	NF	-	+
Solanum nigrum	+	-	-	+
Physalis micranta L.	NF	-	+	-
Schwenckia Americana L.	NF	-	-	-
Portulacacea				
Portulaca oleracea L.	+	-	NF	+
Portulaca quadrifida Linn.	NF	-	-	-
Lythraceae				
Ammannia baccifera ssp. aegyptiaca	-	NF	-	NF
Ceasalpiniaceae				
Cassia occidentalis Linn.	+	+	+	+
Cassia obtusifolia L.	-	+	+	-
Cassia mimosoides Linn.	NF	-	-	-
Convolvulaceae				
Ipomea asarifolia (Desr.) Roem. & Schult.	-	-	-	-
Ipomea eriopcarpa R. Br.	-	-	-	-
Ipomea vagans Bak.	-	-	-	-
Merremia aegyptica (Linn.)	NF	-	NF	-
Malvaceae				
Sida corymbosa R. E. Fries	-	-	-	NF
Sida rhombifolia L.	-	-	-	+
Sida acuta Burm f.	-	-	-	-
Urena lobata Linn.	NF	-	-	-
Tiliaceae				

Corchorus trilocularis L.	+	-	+	+
Cleomaceae				
Cleome viscosa L.	-	-	-	-
Cleome rutidosperma D.C.	-	-	-	NF
Rubiaceae				
Oldenlandia herbacea (Linn.) Roxb.	-	-	NF	-
Oldenlandia crymbosa Linn.	NF	-	+	NF
Spermacoce verticillata Linn.	+	-	-	-
Mitracarpus villosus (Sw.) DC.	+	+	-	+
Diodia scadens Sw.	NF	+	NF	-
Lamiaceae				
Platostoma africanum P. Beauv.	-	NF	-	-
Leonotis nepetifolia (L.) Ait. f.	-	-	-	-
Selonostenum monostachyus	-	-	-	-
Amaranthaceae				
Amaranthus spinosus L.	-	+	-	-
Gomphrena celosioides Mart.	+	-	-	NF
Celosia laxa Schum. & Thonn.	NF	NF	NF	-
Alternanthera pungens H. B. K.	NF	-	NF	-
Fabaceae				
Alysicarpus ovalifolius (Schumach. & Thonn.)	-	-	-	-
Alysicarpus glumaceus (Vahl.) DC.	-	-	-	-
Crotalaria retusa Linn.	NF	-	-	-
Desmodium scorpiurus (Sw.) Desv.	-	NF	NF	-
Indigofera hirsuta Linn. *var. hirsute*	NF	-	-	-
Desmodium tortuosum (Sw.) DC.	NF	-	-	-
Cucurbitaceae				
Luffa aegyptiaca Mill.	-	NF	-	NF
Momordica charantia Linn.	-	-	NF	NF
Acanthaceae				
Hypoestes cancellata Nees.	-	-	NF	-
Nelsonia canescens L.	-	+	-	+
Urticaceae				
Fluerya aestuans (Linn.) ex Miq.	-	NF	NF	NF
Aizoaceae				
Trianthema portulacastrum Linn.	NF	-	-	NF
Onagraceae				
Ludwigia decurrens Walt.	NF	-	-	-

Note: DS = Dry season, WS= Wet season, + = Virus present, - = virus absent, NF= Weed Not found

Table 2: Weed species tested against antiserum of *Tomato ringspot virus* in Jigawa State during 2017 and 2018 dry and wet seasons.

Weed families/species	2017		2018	
	DS	WS	DS	WS
Asteraceae				
Tridax procumbens	NF	-	-	-
Ageratum conyzoides L.	-	-	NF	-
Apilia Africana (Pers) D.C.	-	NF	-	-
Chrysanthelium indicum	NF	NF	-	-
Acanthospermum hispidum DC.	+	-	-	-
Venonia cinerea (Linn) Less	NF	-	-	-
Lactuca serriola	+	+	+	+
Euphorbiaceae				
Euphorbia hirta L.	+	-	-	-
Phyllanthus amarus Schum.	-	-	+	NF
Euphorbia hyssopifolia Linn.	NF	+	-	NF
Euphorbia heterophylla Linn.	-	NF	-	+
Commelinaceae				
Commelina erecta L.	+	+	+	NF
Commelina diffusa Burm. f.	-	-	-	-
Commelina benghalensis L	-	-	+	-
Sterculiaceae				
Melachia corchorifolia Linn.	NF	NF	-	-
Waltheria indica Linn.	-	NF	-	NF
Nyctaginaceae				
Boerhavia erecta L.	-	+	-	NF
Boerhavia diffusa L.	-	-	-	-
Solanaceae				
Physalis angulata L.	+	+	+	NF
Solanum nigrum L.	+	+	+	+
Boraginaceace				
Heliotropium indicum Linn.	NF	-	-	+
Portulacacea				
Portulaca oleracea L.	-	+	+	-

Portulaca quadrifida Linn.	-	-	-	-
Ceasalpiniaceae				
Cassia occidentalis Linn.	+	NF	+	+
Cassia obtusifolia L.	+	-	+	+
Cassia mimosoides Linn.	-	-	+	-
Convolvulaceae				
Ipomea aquatic	+	NF	-	-
Ipomea eriopcarpa R. Br.	-	+	NF	-
Merremia aegyptia (Linn.) Urban	-	-	-	NF
Melastomataceae				
Dissotis rotundifolia (Sm.) Triana	NF	-	NF	-
Malvaceae				
Sida corymbosa R. E. Fries	-	+	-	-
Hibiscus asper Hook f.	-	-	-	NF
Hibiscus sabdarifa	+	-	-	-
Malvastrum coromandelianum (Linn.) Garcke	-	-	-	-
Tiliaceae				
Corchorus trilocularis L.	+	-	-	-
Cleomaceae				
Cleome viscosa L.	-	-	-	-
Cleome rutidosperma D.C.	NF	-	-	-
Cleome monophylla L.	NF	-	NF	-
Rubiaceae				
Oldenlandia herbacea (Linn.) Roxb.	+	-	NF	-
Spermacoce verticillata Linn.	-	-	-	+
Mitracarpus villosus (Sw.) DC.	NF	-	-	-
Richardia brasiliensis Gomez	NF	NF	+	-
Lamiaceae				
Platostoma africanum P. Beauv.	-	-	NF	-
Leonotis nepetifolia (L.) Ait. f.	+	NF	-	-
Selonostenum monostachyus	NF	-	-	NF
Hyptes suaveolens Poit.	+	-	NF	-
Amaranthaceae				
Amaranthus spinosus L.	-	+	+	+

Gomphrena celosioides Mart.	-	-	-	-
Amaranthus viridis	+	-	-	-
Chenopodium album L.	NF	-	-	+
Fabaceae				
Alysicarpus ovalifolius (Schumach. & Thonn.)	-	+	-	NF
Alysicarpus glumaceus (Vahl.) DC.	-	-	NF	-
Desmodium scorpiurus (Sw.) Desv.	-	-	NF	-
Tephrosia flexuosa	+	NF	-	NF
Crotalaria retusa Linn.	NF	-	NF	-
Indigofera hirsuta Linn. *var. hirsute*	+	-	NF	NF
Cucurbitaceae				
Luffa aegyptiaca Mill.	-	NF	-	-
Momordica charantia Linn.	-	-	NF	-
Polygonoceae				
Polygonum lanigenum R. Br.	+	-	NF	NF
Acanthaceae				
Hypoestes cancellata Nees.	NF	NF	-	-

Note: DS = Dry season, WS = Wet season, + = Virus present, - = virus absent, NF = Weed Not found

Table 3: Weed species tested against antiserum of *Tomato ringspot virus* in Kano State during 2017 and 2018 dry and wet seasons.

Weed families/species	2017		2018	
	WS	DS	WS	DS
Asteraceae				
Acanthospermum hispidum DC.	+	+	+	+
Ageratum conyzoides L.	+	+	+	+
Apilia Africana (Pers) D.C.	-	-	-	NF
Bidens pilosa	+	-	+	+
Lactuca serriola	-	NF	-	+
Lactuca virosa	-	+	-	NF
Laggeria aurita	+	+	-	+
Vernonia galamensis (Cass.) Less	NF	-	+	-
Euphorbiaceae				
Euphorbia hirta L.	+	-	NF	-

Phyllanthus amarus Schum.	-	-	-	-
Euphorbia heterophylla Linn.	-	NF	-	+
Jatropha curcas Linn.	-	-	-	-
Commelinaceae				
Commelina diffusa Burm. f.	-	+	-	-
Commelina benghalensis L	-	-	-	-
Commelina erecta L.	-	-	NF	-
Zygophyllaaceae				
Tribulus terestris L.	NF	-	-	-
Solanaceae				
Physalis angulata L.	-	-	-	-
Solanum nigrum	+	+	+	+
Portulacacea				
Portulaca oleracea L.	+	+	-	-
Acanthaceae				
Nelsonia canescens (Lam.) Spreng.	NF	+	+	+
Ceasalpiniaceae				
Cassia obtusifolia L.	-	-	+	+
Cassia mimosoides Linn.	NF	-	-	-
Cassia occidentalis Linn.	-	-	-	-
Convolvulaceae				
Ipomea triloba	NF	-	-	-
Ipomea eriopcarpa R. Br.	-	+	+	+
Malvaceae				
Sida corymbosa R. E. Fries	-	-	NF	-
Hibiscus asper Hook f.	NF	-	NF	-
Malvastrum coromandelianum (Linn.) Garcke				
Urena lobata Linn.	-	-	-	-
Tiliaceae	-	+	NF	-
Corchorus trilocularis L.	-	-	-	-
Onagraceae				
Ludwigia decurrens Walt.	-	NF	-	NF
Rubiaceae				
Oldenlandia herbacea (Linn.) Roxb.	NF	-	-	-

Oldenlandia crymbosa Linn.	-	-	-	NF
Spermacoce verticillata Linn.	-	+	-	-
Mitracarpus villosus (Sw.) DC.	-	-	-	-
Lamiaceae				
Mentha arvensis	-	NF	NF	NF
Leonotis nepetifolia (L.) Ait. f.	-	+	-	-
Amaranthaceae				
Celosia argentica L.	-	-	NF	-
Amaranthus spinosus L.	-	+	+	-
Amaranthus viridis L.	-	-	-	+
Chenopodium album L.	NF	+	+	+
Gomphrena celosioides Mart.	+	+	+	-
Fabaceae				
Alysicarpus glumaceus (Vahl.) DC.	-	-	NF	-
Urticaceae				
Fluerya aestuans (Linn.) ex Miq.	-	NF	-	NF
Moraceae				
Ficus exasperata Vahl.	NF	-	NF	NF

Note: DS= Dry season, WS = Wet season, + = Virus present, - = virus absent, NF = Weed Not found

DISCUSSION

The study reveals the detection of weed species susceptible to ToRSV in their natural environment across tomato fields in Kano, Gombe and Jigawa States of Nigeria. Several reports have documented the successful use of ELISA in detection of plant viruses and their weeds hosts (Chatzivassiliou, *et al.,* 2001; Kashina *et al.,* 2002; Arli-Sokmen *et al.,* 2005; Heydarnejad *et al.,*2007; Massumi *et al.,* 2009; Hajiabadi *et al.,* 2012; Asala *et al.,* 2014; Al-Shahwan *et al.* 2016; Aguiar *et al.,* 2015, 2018; Abraham *et al.,* 2019a). In comparison to previous reports, the current study reports for the first time, the susceptibility of 19 weed species (*Acanthospermum hispidum* DC., *Bidens pilosa* Linn., *Lactuca serriola* L. and *Ageratum conyzoides* L. (*Asteraceae), Ipomea eriocarpa* R. Br. (*Convolvulaceae*), *Gomphrena celosioides* Mart., *Laggeria aurita* (L.f.) DC, *Chenopodium album* L. and *Amaranthus spinosus* L. (*Amaranthaceae*), *Nelsonia canescen* (Lam.) Sprengs (*Acanthaceae*), *Solanum nigrum* L., *Portulaca oleraceae* L. (*Portulacacea*), *Cassia obtusifolia* L. and *Cassia occidentalis* Linn. (*Ceasalpiniaceae*), *Physalis. angulata* L. (*Solanaceae*), *Mitracarpus villosus* (Sw.) DC. (*Rubiaceae*), *Boerhavia diffusa* L. (*Nyctaginaceae)*, *Corchorus trilocularis* L. (*Tiliaceae*), and *Commelina errecta* (*Commelinaceae*)) from 11 families as hosts of ToRSV within and around tomato fields in Nigeria. This finding is consistent with earlier reports (Zitter, 2001; Kashina *et al*., 2002; Al-Shahwan *et al.,* 2016; Aguiar *et al.,* 2018) that documented these weeds as hosts of a number of plant viruses in nature. The prevalence of ToRSV in fields-grown tomato in Kano, Gombe and Jigawa States has been well documented (Abraham *et al.,* 2019b). It was observed in the current study that only six weed species viz: *C.*

occidentalis, A. spinosus, S. nigrum, C. obtusifolia, A. hispidum, and *P. angulate* out of the 19 found to habour the ToRSV developed symptoms such as necrosis, mottling, chlorosis, curling and size reduction of leaves. This finding is in accordance with previous observation by Papayiannis *et al.* (2011) that most weed hosts of viruses are asymptomatic. Such condition ensures the secondary spread of the virus from these symptomless weed hosts to healthy tomato crops. Moreover, further spread of ToRSV could be facilitated through the seeds of *Gomphrena celosioides,* a weed that was found infected in the tomato fields (Sastry, 2013; EPPO, 2016, 2018). Farmers' ignorance about the virus and its vector, all-year-round field cultivation of tomato, persistence nature of weed species and poor weed management in the states where the samples were sourced could be other significant factors influencing the persistence and spread of the virus. *S. nigrum* that had the highest (10.3 %) frequency of weed species infected with ToRSV comparable to other weed host species suggests its high level of susceptibility to both the virus and the virus stability in the weed. *S. nigrum* share the same family with *Solanum lycopersicon* which is a principal host of ToRSV thereby, suggesting that plants from the family *Solanaceae* seem to be more susceptible to the virus.

CONCLUSION

The present study established for the first time the prevalence of ToRSV naturally infecting nineteen weed species within and around tomato fields in northern Nigeria. This finding is relevant towards understanding the epidemiology of the virus and will facilitate effective integrated management practices in the study area. Molecular characterization of the virus to determine the existence of possible strain(s), relationship with other ToRSV species/strains reported elsewhere and for other functional studies is recommended.

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