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Alternative Host Plants (Crops And Weeds) For Groundnut Ringspot Virus (Grsv) In Kenya

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ABSTRACT: *Groundnut Ringspot Virus (GRSV) infects a wide range of plants in the families of Solanaceae, Fabaceae and many others. Most crops Infected; tomato, pepper, eggplant, tomatillo, Nicotiana, groundnut (peanut), soybean, mungbean, peas, watermelon, lettuce and some common garden weeds among others. The symptoms Induced by this virus include; ringspots, leaf mosaic, leaf distortion, leaf chlorosis with green spots, stem necrosis, stunted growth and bronzed with necrotic lessions wilting fruit deformities among others. The virus is transmitted mostly by Frankliniella occidentalis and Frankliniella schultzei Thrips. GRSV and TSWV have similar biological symptoms but differentiated using serological tests. Typical Symptoms for GRSV appears on crops grown by small scale farmers and other plants in western Kenya but no report had been documented on the host plants to the virus in Kenya. The general objective of this study was to determine the host plants to GRSV in both garden crops and weeds growing in Kenya. Survey on prevalence of GRSV, was conducted in short and long rain seasons of the years 2019 and 2020 in western Kenya to identify plant species having typical symptoms of GRSV and their distribution in different ecological zones. Serological analysis was done on samples collected using polyclonal and monoclonal antisera against GRSV and TSWV respectively. Health tested seeds to GRSV of plant species were planted in plastic pots of a mixture of sterilized loam, sand and organic manure at a ratio of 2:1:1 respectively in a greenhouse to screen for their response and host range to GRSV and inoculated with GRSV inoculum. Plants symptomatic development observed at an interval of 5 days for 8 weeks and plant samples for each species collected for GRSV ELISA Tests. Screened plants; Pigeon peas (Cajanus cajan), Bambara nut (Vigna subterranean), Chenopodium album, Galinsoga parviflora, Ageratum conyzoides, American burn weed, Commelina benghalensis, Solanum incanum, Solanum ptychanthum, were revealed for the first time globally being the alternative host for GRSV among others. Most of legumes crops and garden weeds that display typical symptoms of GRSV are alternative hosts for GRSV in Kenya. There is need to avoid intercropping crops that display typical symptom of the virus with desired crops of economic importance. Introgression of resistant genes into crops of economic importance to gain resistance to the virus should be done with urgency to reduce GRSV incidences. Also weeds with typical symptoms of GRSV should be eliminated from crops that are susceptible to GRSV to minimize transmission of the virus to crops.*

KEYWORDS; GRSV, Host plant, symptoms, Inoculum, Screening, antisera, polyclonal.

Introduction

Groundnut ringspot virus infect some legumes, which include groundnuts, Soybeans, and Solanaceae plants; pepper, cucumber, tomatillo and some garden weeds (Webster *et al*., 2011). This virus was first reported on groundnuts and solanaceous vegetables in Peninsular (Adkins *et al*., 2010). Then subsequently detected in tomatoes in southwest and southeast of Florida. Later the virus symptoms were observed on pepper, tomatillo and eggplant (Webster *et al*., 2011). The virus has been reported in South Africa on groundnuts (Arachis hypogeae L.). Samples of Soybeans collected from South Africa tested positive for the virus, although its distribution and symptoms was not reported (Pietersen *et al*., 2002). More research on host range to the virus showed that Double Gleam Mix is among the host plant to *Groundnut ringspot virus* (GRSV). The research carried out in 2009 revealed that GRSV was first detected in tomato (*Solanum lycopersicum*), pepper (*Capsicum annuum*), tomatillo (*Physalis philadelphica*), and eggplant (*Solanum melongena*) in south Florida. *Tomato chlorotic spot virus* (TCSV) was subsequently detected in tomato in south Florida in 2012 and in tomato, pepper, jimsonweed (*Datura stramonium*), and lettuce (*Lactuca sativa*) in Puerto Rico and tomato in the Dominican Republic

3.1 Response of groundnuts and other alternative host to GRSV inoculum

Health tested seeds for GRSV of the selected nine groundnut varieties, tomatoes, soybeans and watermelon were planted in 500 ml plastic pots in sterile soil medium composed of loam soil, manure and sand in the ratio of 2:1:1 in a greenhouse. Each variety/species replicated three times in plastic pots. The plants were arranged in a randomized complete block design (RCBD) in the

greenhouse and inoculated with GRSV inoculum at the rate of 2500 µl /plant with a viral load of 1.033 viral titre from groundnut samples collected from a survey in western Kenya. Health controls for each variety/species, were planted separately in a greenhouse from the inoculated varieties/species to avoid contaminations.

3.2 Determination of alternative hosts to GRSV in Western Kenya

Commonly planted legumes, brassicas and cucurbits exhibiting symptoms similar to GRSV were screened for alternative host to GRSV. Beans, green gram, cowpea, Bambara nut, kales, cabbage, butternut, pigeon peas, black gram and peas were screened for host range for GRSV in western Kenya. Garden broad- leafed weeds commonly found growing or bordering groundnut farms in western Kenya and exhibiting viral symptoms similar to those of GRSV were screened to determine alternative hosts for the virus. Goat weeds (*Ageratum conyzoides*), pigweed (*Amaranthus retroflexus*), wondering Jew (*Commelina bengalensis*), Sodom apples (*Calotropis procera*), black jack (*Biden Pilosa*), African black nightshade (*Solanum ptychanthum*), wild spinach (*Chenopodium album*),White nightshade (*Solanum americanum*), American burn weed (*Erechtites hieraciifolius*), double thorn (*Oxygonium sinuatam*), sweet potato (Ipomoea batatas), Nile trumpet (*Markhamia lutea*) planted in 500ml plastic pots, arranged in RCBD in greenhouse and inoculated as described in section 3.1

3.3 Inoculum preparation and inoculation

Thirty grams of symptomatic leaf sample isolates from the survey, serologically testing positive for GRSV with viral titre of 1.033 and free from other viral contaminations, was grounded using a sterilized pestle and mortar, and with the aid of dust powdered Carborundum 320 grit. Freshly prepared ice-cold 0.01M Potassium Phosphate buffer (K_2HP0_4 + KH_2P0_4), pH 7.0, containing 0.2% Sodium Sulfite and 0.01M Mercaptoethanol (1: 6 [w/v] tissue: buffer), added to the ground tissue, mixed and then transferred to falcon tubes, and allowed to stand for 5 minutes in ice, to settle debris at the bottom of tube. The sap kept on ice, until inoculation completed. The Carborundum dusted on plants under study, acted as an abrasive. The inoculum applied gently on the leaf surfaces at a rate of 2500 µl /plant, using saturated cotton wool swab and excess carborundum and inoculum washed out on the groundnut leaves by spraying gently with sterilized distilled water (Hull, 2009). Hands washed with detergent, before proceeding to the next inoculation, to prevent contamination. The inoculated plants observed on weekly basis for viral symptoms development and recorded. This was repeated for 8 consecutive weeks. Leafy samples of each variety collected and tested by DAS-ELISA for GRSV causal agents.

3.4 Inoculation of groundnut varieties/species and alternative hosts

Groundnut positive isolates of 1.033 viral titre to GRSV, macerated and grounded using a pestle and mortar as described in section 3.3. Groundnut varieties, tomatoes, water melon, soy beans, broad leafed weeds and commonly grown crops, inoculated by gently rubbing 2500 µl of the inoculum/plant on leaves dusted with carborundum respectively apart from healthy controls. After inoculation, excess carborundum on plants, gently removed by spraying with water. Tested plants observed for symptom development 3 days after inoculation and thereafter on weekly basis for 8 consecutive weeks. Data collected included: number of symptomatic plants per variety (disease incidence) and disease severity (using 1-4 scale). Leaf sample collected at 6th week and tested for the virus. DAS - ELISA used as described in section 3.3 and Plants that test positive for GRSV were regarded as susceptible. Viral titre in each variety were, determined by taking the average Spectrophotometric absorbance values (at 405nm) for the positive samples. This was used to grade the resistance levels of different varieties to GRSV, determine alternative host for GRSV and its preference.

4.1 Alternative hosts to GRSV in crops grown in western Kenya

Common crops grown legumes, brassicas and cucurbit in western Kenya; Bambara nut, beans, Cowpeas, black grams, green grams, cabbage, kales, butternuts. These plants showed symptoms of groundnut inoculum that were inoculated (plate. 1).

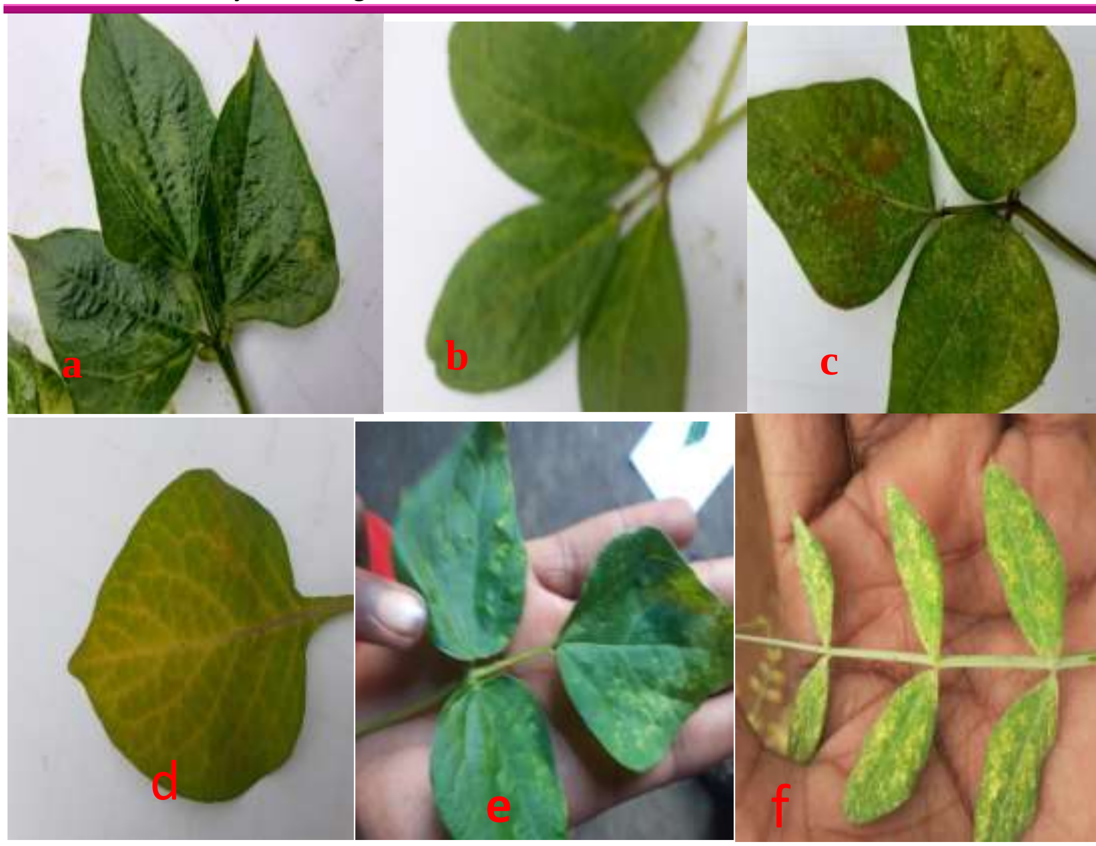

Plate 1. Crops leaves, screened for host range to GRSV. a) cowpea leave with leave mosaic and chlorotic leave spots, b) Bambara nut leaf with leaf mosaic and chlorotic leave veins, c) green gram leaf with chlorotic leaf spots, d) cabbage leaf with chlorotic leaf veins, e) bean leaf with chlorotic leaf spot and leaf mosaic and f) peas leaf with leaf mosaic, these are symptoms of groundnut positive isolates used for inoculation. These plants serologically tested positive for GRSV.

These plants exhibited variant response to GRSV groundnut inoculum after inoculated with positive isolates collected from a survey (Table 1).

Table 1. **Response of Legumes and Brassicaceae crops to GRSV positive isolates**

ID	Name	Family	N	Incidence %	Severity	Symptoms	ELISA
KHRL021	Pigeon peas	Leguminosae	9	14	2	Leaf mosaic, chlorotic leaf spot	+
KHRL021C	Pigeon peas	Leguminosae	3	14	1	No viral disease symptoms	_
KHRL022	Bambara nut	Leguminosae	9	28	3	Leaf mosaic, chlorotic leaf spot, leaf deformation, upward leaf curling	+
KHRL022C	Bambara nut	Leguminosae	3	28	1	No viral disease symptoms	_
KHRL023		Leguminosae	9	0	1	No viral disease symptoms	_

	Green gram						
KHRL024	Black gram	Leguminosae	9	0	1	No viral disease symptoms	_
KHRL024C	Black gram	Leguminosae	3	0	1	No viral disease symptoms	_
KHRL025	Peas	Leguminosae	9	43	3.2	Leaf mosaic, leaf chlorosis, necrotic leaf spot. Leaf vein chlorosis.	+
KHRL025C	Peas	Leguminosae	3	0	1	No viral disease symptoms	_
KHRL026	Cowpeas	Leguminosae	9	21	2.4	Leaf mosaic, chlorotic leaf spot, leaf curling and leaf chlorosis.	+
KHRL026C	Cowpeas	Leguminosae	3	0	1	No viral disease symptoms	_
KHRL027	Beans	Leguminosae	9	0	1	No viral disease symptoms.	_
KHRL027C	Beans	Leguminosae	3	0	1	No viral disease symptoms.	_
KHRL028	Cabbage	Brassicaceae	9	29	2.6	Leaf mosaic, leaf chlorosis, leaf vein chlorosis, necrotic leaf spots	+
KHRL028C	Cabbage	Brassicaceae	3	29	1	No viral disease symptoms	_
KHRL029	Butter nut	Cucurbitaceae	9	40	3.4	Leaf mosaic, chlorotic ringspot, leaf vein chlorosis, necrotic leaf spot and leaf chlorosis	+
KHRL029C	Butter nut	Cucurbitaceae	3	0	1	No viral disease symptoms	_
KHRL030	Kale	Brassicaceae	9	0	1	No viral disease symptoms.	_
KHRL030C	Kale	Brassicaceae	3	0	1	No viral disease symptoms.	_

4.2 Alternative hosts of broad-leafed weeds to GRSV in Western Kenya

Broad-leafed garden weeds commonly growing in farm gardens in western Kenya; goat weeds (*Ageratum conyzoides*), pigweed (*Amaranthus retroflexus*), wondering Jew (*Commelina bengalensis*), Sodom apples (*Calotropis procera*), black jack (*Biden Pilosa*), African black nightshade (*Solanum ptychanthum*), wild spinach (*Chenopodium album*), White nightshade (*Solanum americanum*), American burn weed

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(*Erechtites hieraciifolius*), double thorn (*Oxygonium sinuatam*), sweet potato (Ipomoea batatas), Nile trumpet (*Markhamia lutea*) showed different response and symptoms on the groundnut positive isolates to GRSV (Table 2), (plate. 2) and (Figure. 1).

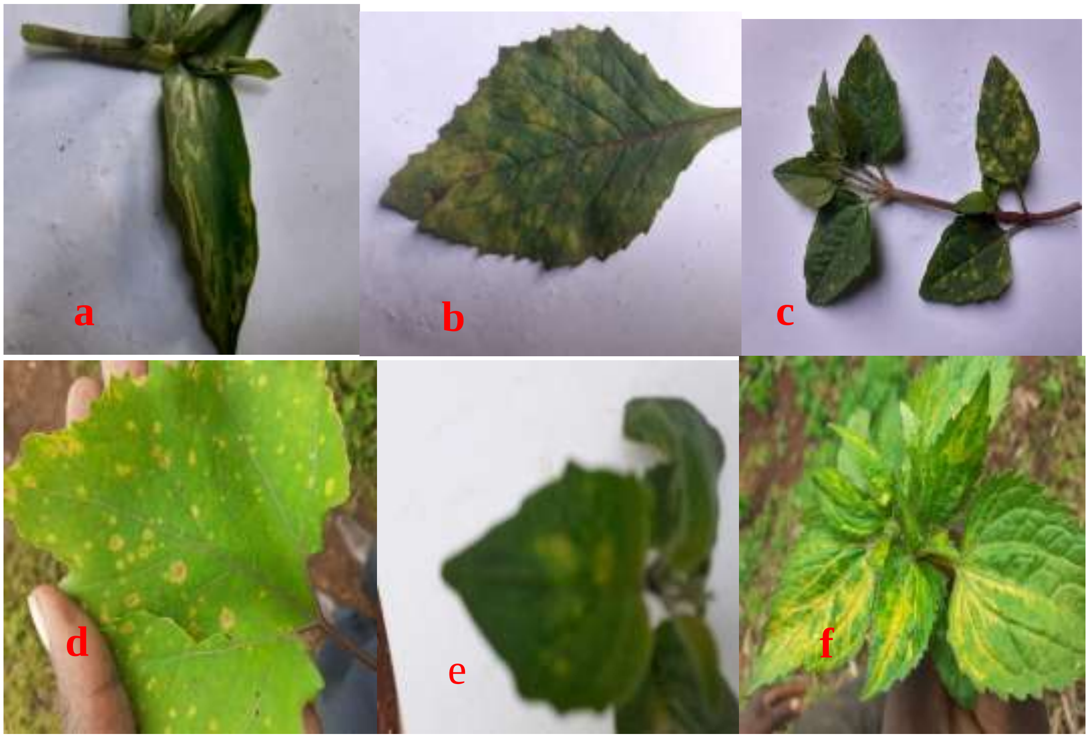

Plate 2. Screened broad-leafed weeds for GRSV host range tested serologically positive for GRSV indicating disease symptom for the virus. a), leaf of wondering Jew with chlorotic ringspots, b) A leaf of American burn weed showing leaf mosaic, c) a leaf of Sphaeranthus indicus with chlorotic spots, d) a leaf of Chenopotum album; with necrotic and chrolotic leaf spots, e) a leaf of solanum americanum with leaf mosaic and f) a leaf of Ageratum conyzoides with leaf chlorosis. The symptoms displayed by these plants was on groundnut innoclum used.

Table 2. Screened broad-leafed weeds for alternative hosts to GRSV

ID	Name	Family	N	Incidence %	Severity	Symptoms	ELISA
KHRW041	Achyanthes bidentate	Amaranthaceae	9	4,8	1	No viral disease symptoms	_
KHRW42	Amaranthus retroflexus	Amaranthaceae	9	26	2	Leaf mosaic,leaf chlorosis and necrotic leaf spot.	+
KHRW43	Bidens Pilosa	Asteraceae	9	5.5	1	No viral disease symptoms	_

KHRW44	American burn weed	Asteraceae	9	55	2.6	Leaf chlorosis, leaf mosaic, leaf deformatin and leaf curling	+
KHRW45	Commelina benghalensis	Commelinaceae	9	28	2	Leaf chlorosis, chlorotic ringspot, leaf mosaic.	+
KHRW46	Datura stramonium	Solanaceae	9	46	3.6	Leaf mosaic, leaf chlorosis, chlorotic leaf spot.	+
KHRW47	Chenopodium album	Amaranthaceae	9	34	3	Leaf necrosis, chlorotic leafspot, leaf deformation and leaf chlorosis.	+
KHRW48	Solanum incanum	Solanaceae	9	43	3.8	Leaf mosaic, leaf chlorosis, necrotic leaf spot.	+
KHRW49	Ageratum conyzoides	Asteraceae	9	0	1	No viral disease symptom	_
KHRW50	Oxygonium sinuatam	Polygonaceae	9	0	1	No viral disease symptoms	_
KHRW51	Solanum Americanum	Solanaceae	9	58	3.4	Leaf vein chlorosis, necrotic leaf spot and leaf deformation.	+
KHRW52	Solanum ptychanthum	Solanaceae	9	59	3.5	Leaf mosaic, leaf chlorosis, necrotic leaf spot.	+
KHRW53	Ipomoea batatas	Convolvulaceae	9	0	1	No viral disease symptoms.	_
KHRW54	Persea Americana	Lauraceae	9	0	1	No viral disease symptoms	_
KHRW55	Markhamia lutea	Bignoniaceae	9	0	1	No viral disease symptoms	_
KHRW56	Amaranthus rudis	Amaranthaceae	9	0	1	No viral disease symptoms	_
KHRW57	Ageratum conyzoides	Asteraceae		26	2.4	Chlorotic leaf spot, necrotic leaf spot	+

KHRW58	Galinsoga parviflora	Asteraceae	9	31	2.6	Chlorosis ringspot, leaf mosaic.	+

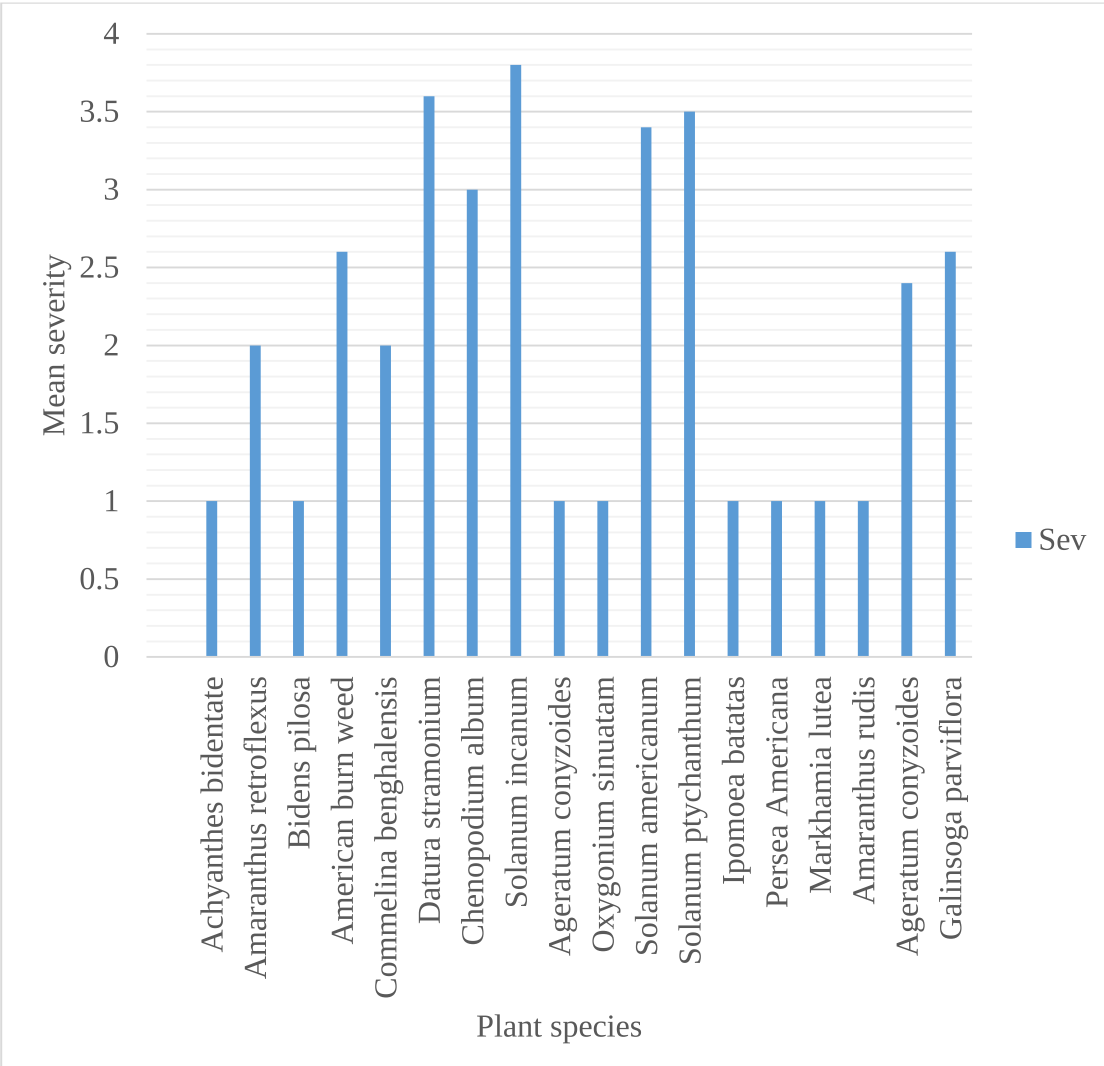

Figure 1: Graph showing variations in mean severity on screened broad-leafed weeds for host range to GRSV in western Kenya

4.3 Response of alternative host to groundnut GRSV positive isolates

Tomatoes, Watermelon, Soybeans and groundnuts responded differently to GRSV inoculum from groundnuts samples. The virus was more virulent to tomato plant than any other host plant screened for response to GRSV groundnut inoculum. Visual symptoms exhibited were chlorotic leaf spot, necrotic leaf spots and leaves with leave deformation, necrotic patches on stems and fruits were also noted. Viral symptoms noted on groundnuts and Soybeans was leaf mosaic and chlorotic leaf spots. Groundnuts than in soybeans. After inoculation, tomatoes exhibited disease symptoms after three weeks; Leaf chlorosis, necrotic parches appeared both on leaves. Groundnuts displayed disease symptoms stem and fruits symptoms were more Disease incidence and severity were observed and recorded also symptom variation and development were recorded progressively (plate.3), (Table.3) and (Figure.2) development were recorded progressively (plate.3), (Table.3) and (Figure .3).

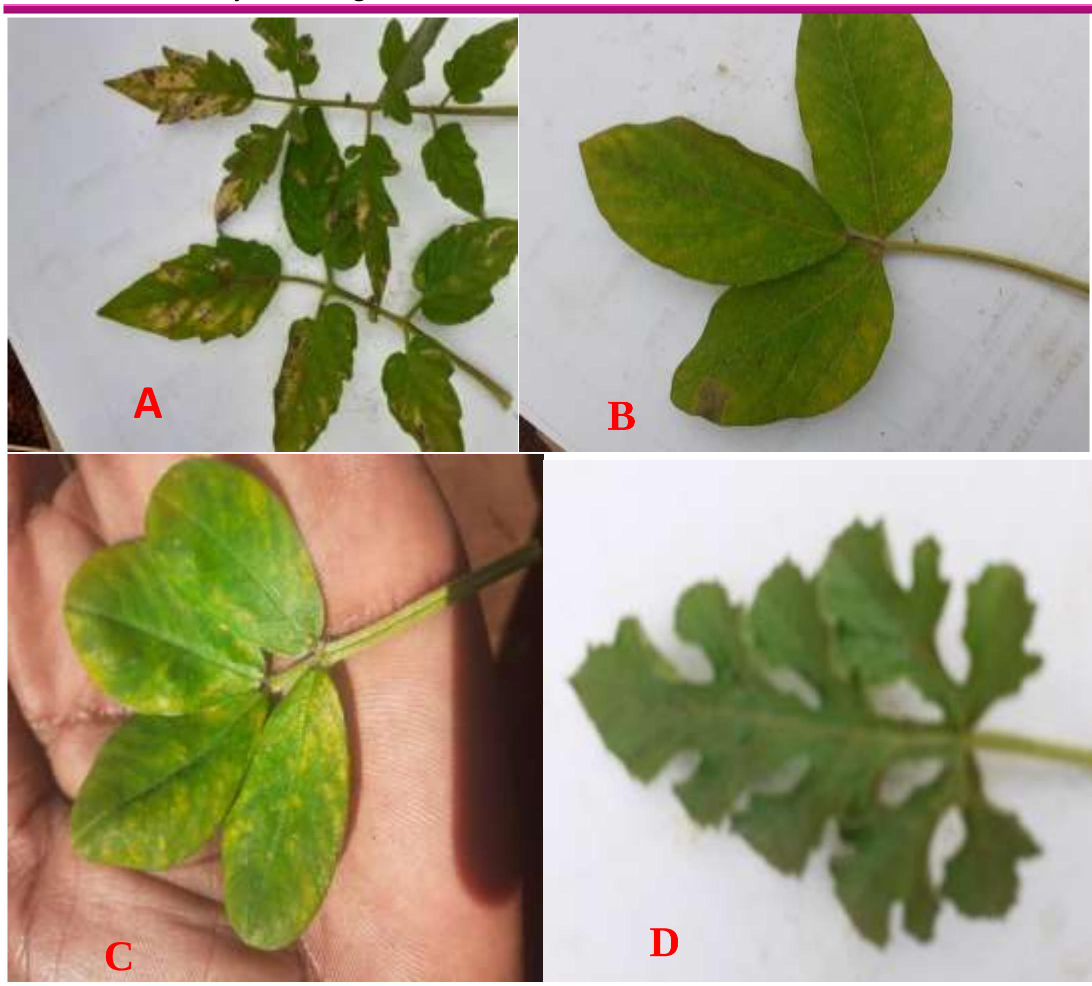

Plate 3. Showing visual symptoms of screened host plants to GRSV in response to groundnut positive inoculum isolates. A) a leaf from tomato plant with necrotic and chlorotic patches, B) a leaf from Soy bean with leaf mosaic and necrotic leaf spot, C) a leaf from groundnut with leaf mosaic and chlorotic leaf spots and D) a leaf from watermelon with no disease symptoms.

Table 2: Symptomatic, ELISA tests and scores for alternative hosts to GRSV

ID	Species	Family	N	Incidence	Severity	Symptoms	ELISA
HR91	Tomato	Solanaceae	6	80	3.8	Leaf chlorosis, upward leaf curling, necrotic sperks on stems	+
HR91C	Tomato	Solanaceae	3	0	1	Absence of viral disease symptoms	_
HR92	Watermelon	Curcurbitaceae	6	0	1	Absence of viral disease symptoms	+

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HR92C	Watermelon	Curcurbitaceae	3	0	1	Absence of viral disease symptoms	_
HR93	Soy beans	Leguminosae	6	32	2.4	Necrotic leaf spot, leaf mosaic	+
HR93C	Soy beans	Leguminosae	3	0	1	Absence of viral disease symptoms	_
HR94	Groundnuts	Leguminoseae	6	64	3.2	Chlorotic ringspot,necrotic spots,leaf mosaic, reduced height.	+
HR94C	Groundnuts	Leguminoseae	3	0	1	Absence of viral disease symptoms	_

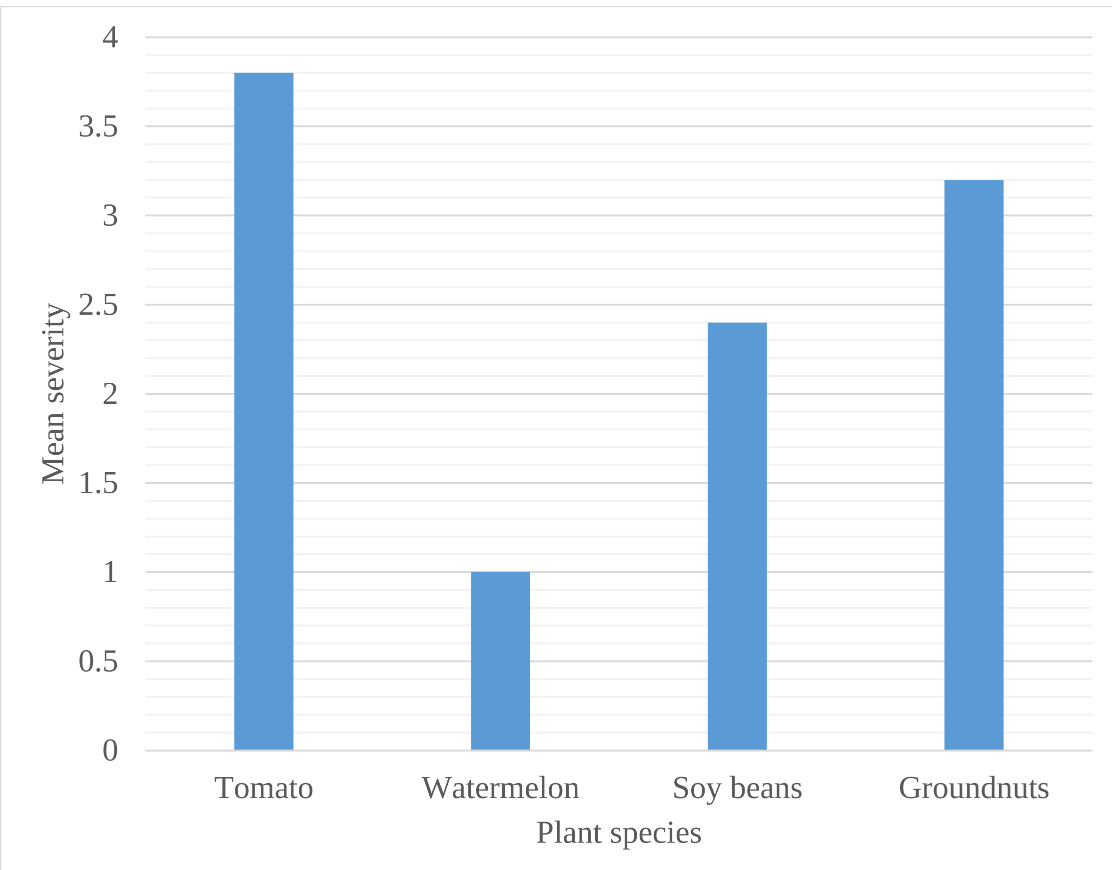

Figure 3: Graph of variations in severity in screened host plants in response to GRSV to positive isolates inoculum from serologically tested positive isolates of groundnuts in western Kenya.

5.0 Conclusion and Recommendation

Response of screened tomatoes to GRSV groundnut inoculum was higher (80 %) than groundnuts (64 %) and Soyabean (32 %). Watermelon displayed no viral symptoms and tested negative for the virus by ELISA (Webster *et al*., 2015). Implying that tomatoes are more susceptible to GRSV groundnut strains than groundnuts and soybeans. This may be, tomatoes lack RNA Antiviral silencing gene mechanism to defend the crop from being attacked by the virus (Sheikh *et al*., 2018). Soybeans displayed viral symptoms, which is contrary to the report (Pietersen *et al*., 2002), indicating that soybeans show no disease symptoms but serologically test positive for

GRSV. Watermelon was asymptomatic but serologically tested positive for the virus. This may indicate that watermelon is tolerant to GRSV strains of groundnut inoculum (Maksimov *et al*., 2019). Among broad leafed-weeds, screened for alternative hosts to GRSV; *Amaranthus retroflexus, American burn weed, Commelina benghalensis, Datura stramonium, Chenopodium album, Solanum incanum, Solanum americanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* exhibited GRSV symptoms and serologically tested positive for the virus (Webster *et al*., 2015). This implies that these weeds are alternative host for the virus (Boari *et al*., 2002). This study has revealed; American *burn weed, Commelina benghalensis, Chenopodium album, Solanum incanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* for the first time being among the alternative host for virus. Such weeds should completely be eliminated from groundnut farms or other crops of economic importance that are alternative host for the virus (Wisley *et al*., 2005). These weeds when left in farms act as primary inoculum on which thrips pick GRSV strains for transmission to other host plants (Webster *et al.*,2015), thus posing great threat not only to groundnuts but also other crops of economic importance (Webster *et al*., 2011). Some of crops of economic importance mainly intercropped with groundnuts or planted adjacent to groundnut farms in western Kenya; pigeon peas, Bambara nut, green gram, black gram, peas, cowpeas, beans, cabbage, butternut and kales are among the alternative hosts to Groundnut ringspot virus. Also some broad-leafed weeds growing in groundnut farms which had not been reported as alternative host for the virus; American *burn weed, Commelina benghalensis, Chenopodium album, Solanum incanum, Solanum ptychanthum, Ageratum conyzoides and Galinsoga parviflora* are among are alternative hosts to GRSV

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