

Full Length Research Paper

Seed health tests of traditional leafy vegetables and pathogenicity in plants

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Traditional leafy vegetables play a significant role in the daily diets of many people in Zimbabwe. They are produced by smallholder farmers with limited knowledge on the agronomic value of high-quality seed, on seed storage, and on plant- and seed-borne pathogens. The quality of the seed is rarely tested for seed-borne pathogens posing risks of pathogen build-up. This study was conducted in order to determine the seed-borne pathogens associated with traditional leafy vegetable seeds. Seeds were collected from five different farmers in three provinces of Zimbabwe and tested for the presence of pathogens in the plant pathology laboratory of the University of Zimbabwe. A total of 154 fungal and 233 bacterial infections were recorded on 450 seeds for each type of pathogen. Significantly high percentages of the isolates (*Xanthomonas campestris*, *Curvularia* spp., *Alternaria alternata*, *Fusarium oxysporum* and *Pseudomonas syringae*) were obtained from the three different species of traditional leafy vegetables (*Amaranthus hybridus*, *Bidens pilosa* and *Cleome gynandra*). *F. oxysporum*, *A. alternata*, *X. campestris* and *Bacillus* spp. isolates were pathogenic to traditional leafy vegetable plant species as confirmed by pathogenicity tests, hence may have significant effects on traditional leafy vegetables production. The results indicate that seed sourced from farmers 1, 2, 3 and 4 resulted in seed-borne pathogens on both the seed and plants, clearly showing that seed quality was poor. Although most pathogens observed have a broad host spectrum, pathogenicity and virulence tests confirmed that some pathogens were likely to be closely associated with these traditional leafy vegetable seed species.

Key words: Seed-borne pathogens, disease occurrence, pathogenicity, bacteria, fungi, seed quality.

INTRODUCTION

Traditional leafy vegetables (TLVs) of Africa refer to plant species, which originated on the continent and have a long history of cultivation, domestication and use in African conditions (Ambrose-Oji, 2009). *Amaranthus* spp. combine both social and socio-economic importance in

Kenya and Tanzania as a multi-purpose vegetable with environmental adaptability, nutritional, medicinal and income generation properties (Weinberger and Msuya, 2004; Abukutsa-Onyango, 2007), and have gained popularity also in Zimbabwe. Mukwereza (2002) reported

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spider flower as the most preferred TLV in Zimbabwe. Together with *Bidens pilosa* and *Cleome gynandra* (spider flower) *Amaranthus spp.* also combine resilience to climate change, nutritional, medicinal and income generation properties.

Across Africa, TLVs are produced and marketed by smallholder farmers (Ngugi et al., 2007) with limited access to advanced agro-technology including limited knowledge of the agronomic value of high-quality seed (Biemond et al., 2012), lack of information on seed storage (Cernansky, 2015), on appropriate use of chemicals like pesticides and fertilizers (Schippers, 2000), plants and seed-borne pathogens. These smallholder farmers obtain most of their seeds from their neighbours or informal seed markets (Ngwerume and Mvere, 2003; Adebooye et al., 2005; Guei et al., 2011).

This seed is most likely to be of poor quality. Seed is rarely tested for germination and seed-borne pathogens, increasing risks of seed degeneration across generations. Seed-borne pathogens can prevent germination and cause serious threats to seedling establishment (Valkonen and Koponen, 1990; Walcott, 2003) and plant vigour (Anjorin and Mohammed, 2014). Farmers using poor-quality seed risk poor germination and the ultimate crop stand in their fields will be poor. This may also affect crop yields and the quality of their vegetables on the market (Biemond et al., 2012; Mancini and Romanazzi, 2013).

Most TLVs are propagated by seed (Grubben and Denton, 2004). However, seed is the most important vehicle for transmitting plant pathogens globally (Kuan, 1988). Plant pathogens are reported to have close associations with seed, as they can survive in, on or with seed, hence can be preserved in seed lots (Copeland and McDonald, 2001), at times in latent stage, are temperature dependent (Ngadze and Icishahayo, 2014), and manifest themselves and become problematic only when environmental conditions become conducive for their growth and reproduction (Copeland and McDonald, 2001). Seed has the potential to carry a wide range of pathogens including fungi, bacteria, viruses, many of which cause diseases in various plant developmental stages (from seedling to the product on the markets) (Copeland and McDonald, 2001).

A seed health test is an important component for determining the quality of seed worldwide, a prerequisite to minimise losses by assessing the quality of seed before it is sown (Copeland and McDonald, 2001). Seed health tests have been done on various seeds including tree seed, vegetable seed, and cereal seed (International Seed Testing Association - Seed Health (ISTA), 2015). Relatively very limited research on TLV seed has been reported (Sharma et al., 1980) including research on seed health tests, posing risks with seed germination (Cernansky, 2015). Plants are frequently left in the field for too long, risking seed-borne pathogens accumulation on seed (Adebooye et al., 2005).

TLV seed is affected by several seed-borne pathogens such as *Rhizoctonia spp.*, *Sclerotinia spp.*, *Pythium spp.*, *Choanephora cucurbitarum*, *Alternaria spp.*, *Mycoplasma*, *Cercospora spp.*, *Sphaerotheca fuliginea*, and *Oidiopsis taurica* (Sharma et al., 1980; Grubben and Denton, 2004), and some TLVs are important hosts of *Xanthomonas campestris pv. campestris* (Bradbury, 1986), which is also an important seed-borne pathogen of many plant species.

Economically, important examples of these seed-borne pathogens include *Pythium spp.*, *Pythium aphanidermatum* and *Rhizoctonia spp.* causing a serious damping off problem in the *Amaranthus hybridus* seedbed, *Cercospora bidentis* and *Cercospora uramensis* affecting *Bidens pilosa* and *C. gynandra*, respectively (Grubben and Denton, 2004). *C. gynandra* seed is also affected by a range of seed-borne fungi including *Sphaerotheca fuliginea* and *Oidiopsis taurica*. These pathogens were reported to cause powdery mildew on *C. gynandra* plants (Grubben and Denton, 2004).

In Zimbabwe, over 75% growers of TLVs collect and retain seeds for sowing in the next season (Schippers, 2000) and the quality of the seed is rarely known. Moreover, farmers rely on traditional knowledge that is passed on through generations and often use traditional cropping technologies (Schippers, 2000). Considering that most of the seed-borne pathogens affecting TLV seed including *X. campestris*, *Cercospora spp.*, *S. solani* and *Pythium spp.* cause serious problems in vegetable production in Zimbabwe (Handiseni et al., 2008), there is, therefore, a high risk of seed-borne infections. Moreover, Rothwell (1982) listed *Entyloma bidentis*, *Sclerotium rolfsii*, *Sphaceloma spp.*, *Uromyces bidenticola*, *Verticillium dahliae*, *Pseudomonas solanacearum* and *Oidium spp.* as pathogens that affect *Bidens pilosa*, *Amaranthus spp.* and *Cleome spp.* in Zimbabwe. Primary inoculum can also arise from other sources like contaminated tools and equipment (Jones et al., 1986), some TLVs are important host of *Xanthomonas campestris pv. campestris* (Bradbury, 1986), plant debris (Barak and Liang, 2008). Nevertheless, seed-borne inoculum remains paramount importance for most pathogen infections (Naseri and Mousavi, 2015).

Generally, seed pathogens transferred to plants during growth cause considerable loss of crop yields globally (Copeland and McDonald, 2001), but limited literature on the economic losses caused by these pathogens on TLVs have been reported. For example, Ngadze (2014) reported that some plant diseases may affect the plants during the growing phase and later stages in storage as well. The pathogens which are seed-borne affect the whole TLV food chain.

Initially starting with pathogen infected seed, the pathogens may result in poor crop stand due to poor germination or poor-quality seedlings which cannot withstand relative environmental changes- dying away of

plants. In storage and on the markets, the quality of the product is also affected by the pathogens including those passed through the use of infected seed-borne. This may result in food and nutrition insecurity and loss of incomes to the farmers, if little attention is paid to controlling seed-borne pathogens.

The research aims at detection and identification of seed-borne pathogens on TLV seed and establishes the relationship between seed infection and pathogen which develop on the plants in the field. In addition, the study seeks to ignite researches on the diseases and their economic impact on TLV seed. The specific objectives are:

- (1) To identify seed-borne pathogens in TLV seed from different sources;
- (2) To investigate whether pathogens found on seed are pathogenic to the plants.

The main research question is:

- (1) Do seed-borne infections contribute to the disease symptoms observed in the field?

Therefore the hypotheses tested include:

- (1) Seed-borne pathogens' presence varies among seed sources;
- (2) Seed-borne pathogens cause the diseases observed on the leaves of infected plants.

MATERIALS AND METHODS

The study uses three traditional leafy vegetable seed namely *C. gynandra*, *B. pilosa* and *A. hybridus* as examples. *C. gynandra* and *A. hybridus* are C_4 plants (Imbamba et al., 1977; Schippers, 2000), while *B. pilosa* is a C_3 plant. Traditional leafy vegetables have a significant role in the traditional diets of many people in Zimbabwe.

Origin of the seed sources

Seed of the three species was sourced from five different farmers. These farmers were chosen using a snow ball sampling technique, with the entry point as the traditional leafy vegetable markets. Only farmers who had seed stocks from the previous season showing that they had been producing TLV seed were chosen. About 500 g of seed for each species was sourced from the farmers and used for seed health tests. One farmer was a government research institute, that is, Horticultural Research Centre (HRC). Lot sampling was done for seed sourced from HRC because they had bigger quantities in their stock than required and hand sampling was used to come up with approximately 500 grams of seed per each species. For the remaining other seed sources, all the seed available was sourced and most of the seed lots weighed less than 1 kg per species per source. The seed collection consisted of 15 samples (three samples of three species per seed source). Subsampling to approximately 400 seeds per sample was done using the hand halving method (International Seed Testing Association (ISTA), 2016). The seed was sourced from three different agro ecological regions of Zimbabwe: Mashonaland Central, Mashonaland West and Mashonaland East. These

provinces (agro ecological regions) are some of the major vegetable growing ones of Zimbabwe, because of their proximity to major cities and because they are located in areas of high agricultural potential. Our research used only five seed sources and intended to ignite more research on these vegetables across Zimbabwe. The climatic conditions during crop growth and geographical coordinates of the seed origin sites are presented in Table 1.

Detection of seed-borne pathogens

Seeds of the three TLV types were randomly selected and 150 seeds of each type of pathogen were plated on potato dextrose agar (PDA) and nutrient agar (NA) for isolation of fungi and bacteria respectively. The seeds were surface sterilized in 1% sodium hypochlorite and rinsed three times with sterile distilled water. The sterile seeds were blotted with sterile filter paper (Whatman no.1) to remove excess water prior to plating. A total of 10 seeds were plated out on a 90 mm diameter petri dish. Seeds for fungal identification were plated on PDA while those for bacteria were plated on NA plates. Each Petri dish was replicated 6 times (3 plates bacteria and 3 for fungi). The Petri dishes with seeds on PDA were incubated at $25\pm 2^\circ\text{C}$ under 12/12 alternating cycles of NUV and darkness in Plant Pathology incubation room at the University of Zimbabwe for seven days. The seeds on NA were placed in an incubator (Gallenkemp- Sussex, England) and maintained at $30\pm 2^\circ\text{C}$ for 4 days. After incubation the seeds were viewed under a stereo microscope using the 16 $\times$ objective.

Identification of fungi

After the incubation period the fungi, which grew on the seeds, were examined and identified under stereomicroscope (WILD M3B, Heerbrugg, Switzerland) based on the growth characters of the fungi. Slides were prepared using the associated fungi on seeds for identification under a compound microscope (JENA LABOVAL, Göttingen, Germany) for better identification following the keys described by Mathur and Olga (2001) and Commonwealth Mycological Institute descriptions.

Identification of bacteria

After the incubation period, further plating was done on the culture media, colonies of bacteria purified on the solidified potato dextrose agar or nutrient agar, respectively, were then incubated at 25°C for 48 h. The morphological identification of the spores for fungi and bacteria was performed according to Mathur and Olga (2001) and Schaad et al. (2001). Single colonies of each isolate were used for physiological and biochemical tests. The isolates were identified by standard bacterial methods based on Cother and Sivasithamparam (1983). The tests performed were Gram reaction, oxidase activity, glucose metabolism, pectate degradation in Sutton's medium, production of phosphatase, indole production from tryptophan, gelatine hydrolysis, production of reducing substances from sucrose, production of acid from α -methylglucoside and trehalose, malonate utilisation, sensitivity to erythromycin (15 μg), growth at 37°C which was determined after 24 h in nutrient broth (NB, Difco) and salt tolerance which was checked after 48 h growth in NB with 5 g l^{-1} NaCl.

Detection and identification of pathogens on infected plants

The aim was to investigate whether seed-borne pathogens on seed corresponded to disease symptoms observed on

Table 1. Geographical coordinates and climatic data during crop growth of the origin sites of the seed.

Farmer	Origin	Agro ecological region ^a	Mean annual temperature	Temperature range during crop growth	Geographical coordinates	Altitude (m)	Annual rainfall (mm)	Amount of rainfall during crop growth (mm)
1	Mashonaland West	2B	22-29°C	31-22°C	17°22'00" S: 30°11'59" E	1153	700-800	73-0.31
2	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S: 31° 47' 2.40" E	1400	800-850	100-0.8
3	Mashonaland East	3	18-29°C	31-21°C	17° 38' 35.59" S: 31° 47' 2.40" E	1400	800-850	100-0.8
4	Mashonaland Central	3	21-31°C	31-21°C	17° 18' 56" S: 31° 34' 14" E	953	800-850	110-1.5
5	Mashonaland East	2B	18-29°C	29-27°C	18° 11' 6.97" S: 31° 33' 6.95" E)	1688	850-900	105.7-24.8

Note: Agro ecological classification also known as natural region (NR) classification of Zimbabwe, divided the country into five regions based on mean annual rainfall and was done in the 1960s (Vincent and Thomas, 1960).

Source: authors, <https://www.worldweatheronline.com> Historical average weather 20/04/2017 (for full details Annex 1 and 2 description).

plants. Plants were grown in the greenhouse at the University of Zimbabwe in February of 2017. A randomized complete block design with three blocks was used. The pots were labelled and randomly arranged and filled to approximately 6 cm under the rim. The soil was sterilised at 70°C for 24 h and allowed to cool. A mixture of soil and pine bark at ratio 1:3 was done to reduce soil compaction and placed in pots. Proper levelling of the growing media was done before approximately 1000 seeds were evenly distributed per pot. The seeds were then covered with 1.5 cm thick layer of the growing media. Water as needed was added to the pots. The presence of the diseases on plants was also recorded in the greenhouse (for example, damping off).

The infected plant parts showing typical symptoms of diseases were collected for further analysis. Plant pathogens were isolated from different parts of the crops that had advanced lesions and rots. The lesions and surrounding healthy tissues were cut into small pieces and washed in running tap water before sterilizing with 1% sodium hypochlorite for 2 to 3 min, followed by rinsing three times in sterile distilled water, and drying on sterile blotter papers. Disinfected pieces were placed on solidified Nutrient Agar (NA) medium (Schaad et al., 2001). The Petri dishes were incubated at 28±2°C and observed for bacterial growth 4 to 6 days later. After the incubation period, the further plating was done on the culture media,

colonies of fungi and bacteria were purified on the solidified potato dextrose agar or nutrient agar, respectively, and were then incubated at 25°C for 48 h. Thereafter, the morphological identification of the spores for fungi and bacteria was performed according to Mathur and Olga (2001) and Schaad et al. (2001).

Hypersensitivity

Isolated colonies of different bacteria were tested for ability to induce a hypersensitive response on foliage of the non-host plant tobacco (*Nicotiana tabacum* cv. Burley). Five isolates were selected for each of the three bacteria species isolated from seed and infected plants. Test isolates were freshly grown on NA at 30±2°C for 24 h and suspended in sterile water, maintain an inoculums concentration of 10⁸ cfu/ml (using Genie spectrophotometer at 550 nm). Tobacco seedlings were inoculated with suspensions of the selected isolates at the 5 to 6 leaf stage. 1 ml of bacterial suspension was injected using a disposable syringe into the lower surface of the tobacco leaves and the plants were sealed in plastic bags for 24 h to prevent desiccation. Plants were uncovered and kept in the laboratory at 28±2°C for 36 h. Control plants were injected with sterile distilled water and kept under same conditions.

Virulence test

For the assessment of pathogenicity of the different bacteria on TLV seedlings, inoculum suspensions were prepared in sterile water as previously described for the hypersensitive test. Young TLV seedlings were inoculated with 1 ml of inoculum using a hypodermic syringe. Control leaves were inoculated with sterile distilled water.

Data analysis

Data were analysed using GenStat 18th edition. The data were transformed using LOG10 plus 1 and subjected to analysis of variance (ANOVA) and the means of the parameters were separated using the Duncan's multiple range least significant difference test ($P \leq 0.05$). The transformation was done to normalise the data sets because some data sets had many zeros.

RESULTS

A total of 154 fungal and 233 bacterial infections were recorded on 450 seeds (for each type of pathogen) selected from seeds of three types

Table 2. Hypersensitive responses of the different isolates tested on tobacco.

Isolate	Source	Hypersensitive response (+/-)	Duration (hrs)	Virulence
<i>Xanthomonas campestris</i> 1	Seed	+	24	High
<i>Xanthomonas campestris</i> 2	Plant	+	36	Weak
<i>Xanthomonas campestris</i> 3	Seed	+	36	High
<i>Xanthomonas campestris</i> 4	Seed	+	24	Moderate
<i>Xanthomonas campestris</i> 5	Plant	+	72	High
<i>Pseudomonas syringae</i> 1	Seed	+	36	Weak
<i>Pseudomonas syringae</i> 2	Seed	+	24	Weak
<i>Pseudomonas syringae</i> 3	Plant	+	48	High
<i>Pseudomonas syringae</i> 4	Plant	+	72	High
<i>Pseudomonas syringae</i> 5	Plant	+	72	High
<i>Bacillus</i> spp. 1	Seed	+	24	Moderate
<i>Bacillus</i> spp. 2	Plant	+	72	Weak
<i>Bacillus</i> spp. 3	Seed	+	72	High
<i>Bacillus</i> spp. 4	Seed	+	24	High
<i>Bacillus</i> spp. 5	Plant	+	36	Weak
Control	Distilled water	-	24-72	-

of TLV. The fungi were identified based on colony morphologies as *Curvularia* spp., *Alternaria alternata*, *Fusarium oxysporum*, *Aspergillus* spp., *Phoma betae*, *Colletotrichum* spp. and *Rhizoctonia solani*. Three bacteria species were isolated from the seed and they were identified as *Xanthomonas campestris*, *Pseudomonas syringae* and *Bacillus* spp. The seed-borne infections varied according to seed species and source. The virulence test conducted on young TLV seedlings varied by pathogen species and seed species.

Hypersensitivity test

Table 2 shows hypersensitivity responses of the different isolates tested on tobacco. Ten of the fifteen isolates tested on tobacco plants induced a hypersensitivity test within 24 to 48 h after inoculation, and based on these reactions they were categorized as strongly, moderately, or mildly virulent. All isolates were considered as pathogenic since they produced a reaction within 72 h after inoculation. *X. campestris* isolates 1, 3 and 5, *P. syringae* isolates 3, 4 and 5 as well as *Bacillus* spp. Isolates 3 and 4 were regarded as highly virulent. Two isolates *X. campestris* 4 and *Bacillus* spp. 1 were moderately virulent while isolates *X. campestris* 2, *Pseudomonas syringae* 1 and 2 as well as *Bacillus* spp. 1 and 5 were considered to be weakly virulent.

Virulence tests

All isolates gave a positive response on all three TLV plant species (Table 3). The isolates were ranked as

highly, moderately and weakly virulent pathogens based on host responses. Among these highly virulent isolates, symptoms in plants inoculated with *X. campestris* isolates 1, 3 and 4 were highly virulent on *B. pilosa* and *C. gynandra*, and moderately virulent on *Amaranthus hybridus* and the symptoms developed earlier than with other isolates. Plants inoculated with these isolates showed disease symptoms within 6 days. *Pseudomonas syringae* isolates 3 and 4 were highly virulent on *B. pilosa* and *A. hybridus*. No symptoms developed in controls treated with sterile water.

Isolation of pathogens from seed by plant species and source

The percentage occurrence of seed-borne pathogens for plant species and seed farmers were evaluated and are presented in Tables 4 and 5, respectively. For both cases, the percentage occurrence refers to the frequency of any single isolate observed on each seed which is measured as the proportion of the seeds per species that are infected by seed-borne pathogens. The percentage occurrence was calculated by adjusting Jager et al. (2007) calculations as number of seeds having the pathogen during the analysis divided by the total number of seeds used per species and the ratio is multiplied by 100 (following formula used) and subsequently used to determine the differences by performing the analysis of variance and separation of means using Duncan's multiple range method in GenStat.

$$\text{Percentage occurrence} = \frac{\text{No. of seeds affected by a pathogen}}{\text{Total no. of seeds}} \times 100$$

Table 3. Virulence of the different isolates on seedlings of traditional leafy vegetables.

Isolate	<i>Amaranthus hybridus</i>	<i>Cleome gynandra</i>	<i>Bidens pilosa</i>
<i>Xanthomonas campestris</i> 1	++	+++	+++
<i>Xanthomonas campestris</i> 2	++	+	+
<i>Xanthomonas campestris</i> 3	++	+	+++
<i>Xanthomonas campestris</i> 4	++	+++	+++
<i>Xanthomonas campestris</i> 5	++	+++	++
<i>Pseudomonas syringae</i> 1	++	++	+
<i>Pseudomonas syringae</i> 2	++	+	++
<i>Pseudomonas syringae</i> 3	+++	++	+++
<i>Pseudomonas syringae</i> 4	+++	++	+++
<i>Pseudomonas syringae</i> 5	++	++	++
<i>Bacillus</i> spp. 1	+++	+++	++
<i>Bacillus</i> spp. 2	+	++	+++
<i>Bacillus</i> spp. 3	+	++	+
<i>Bacillus</i> spp. 4	+++	+	++
<i>Bacillus</i> spp. 5	+++	++	+++
Control	-	-	-

Note: +++, ++ and + denote highly, moderately and weakly virulent pathogens, respectively.

Table 4. The percentage occurrence of seed-borne pathogens on seed for three plant species (between brackets the transformed values and the mean separation).

Name of pathogen	Plant species			LSD _{5%}
	<i>Amaranthus hybridus</i>	<i>Cleome gynandra</i>	<i>Bidens pilosa</i>	
Bacteria				
<i>Xanthomonas campestris</i>	1.3 (0.09 ^a)	79.3 (1.60 ^b)	58.7 (1.20 ^b)	0.57
<i>Pseudomonas syringae</i>	6.7 (0.36 ^{ab})	0.0 (0.00 ^a)	8.7 (0.53 ^b)	0.40
<i>Bacillus spp.</i>	0.7 (0.07 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.11
Fungi				
<i>Alternaria alternata</i>	1.3 (0.14 ^a)	18.7 (1.12 ^c)	10.7 (0.74 ^b)	0.38
<i>Aspergillus spp.</i>	0.0 (0.00 ^a)	0.0 (0.00 ^a)	4.7 (0.19 ^a)	0.22
<i>Fusarium oxysporum</i>	12.0 (0.57 ^a)	3.3 (0.18 ^a)	1.3 (0.14 ^a)	0.41
<i>Curvularia spp.</i>	2.7 (0.23 ^a)	25.3 (0.78 ^a)	18.7 (0.69 ^a)	0.55
<i>Phoma betae</i>	0.0 (0.00 ^a)	0.0 (0.00 ^a)	1.3 (0.09 ^a)	0.15
<i>Colletotrichum spp.</i>	0.0 (0.00 ^a)	1.3 (0.14 ^a)	0.0 (0.00 ^a)	0.16
<i>Rhizoctonia solani</i>	1.3 (0.09 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.15

The mean separation indicates per pathogen the differences among the three species ($P \leq 0.05$).

Table 4 shows the percentage occurrence of seed-borne pathogens isolated from seeds of three species. In comparison among plant species, highest percentage occurrence of seed-borne pathogens was observed on *C. gynandra*, followed by *B. pilosa* and least on *A. hybridus* seed. Only *X. campestris*, *P. syringae* and *A. alternata* isolates were significantly different for the percentage occurrence of seed-borne pathogens on seed among the three plant species.

For *X. campestris*, the percentage occurrence of seed-borne pathogens on seed for *C. gynandra* and *B. pilosa*

did not differ from each other, which was significantly higher than for *A. hybridus*. On the other hand, *C. gynandra* showed the lowest percentage occurrence on *P. syringae*. For both plant species, the percentage occurrence of *A. alternata* on seed was highest on *C. gynandra*, intermediate on *B. pilosa* and lowest on *A. hybridus*.

Table 5 shows the percentage occurrence of seed-borne pathogens per farmer. The percentage occurrence of a single bacterial or fungal isolate differed among the farmers but only significantly for *Curvularia* spp. and

Table 5. The percentages occurrence of seed-borne pathogens per seed farmer (between brackets the transformed values and the mean separation).

Name of pathogen	Seed source					LSD 5%
	Farmer 1	Farmer 2	Farmer 3	Farmer 4	Farmer 5	
Bacteria						
<i>Xanthomonas campestris</i>	35.7 (0.82 ^a)	64.4 (1.33 ^a)	66.7 (1.34 ^a)	33.3 (0.67 ^a)	32.2 (0.66 ^a)	0.95
<i>Pseudomonas syringae</i>	3.3 (0.17 ^a)	2.2 (0.15 ^a)	7.8 (0.44 ^a)	6.7 (0.33 ^a)	5.6 (0.41 ^a)	0.56
<i>Bacillus spp.</i>	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	1.11 (0.12 ^a)	0.15
Fungi						
<i>Alternaria alternata</i>	7.8 (0.64 ^a)	16.7 (0.93 ^a)	10.0 (0.67 ^a)	10.0 (0.58 ^a)	6.7 (0.53 ^a)	0.64
<i>Aspergillus spp.</i>	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	6.7 (0.20 ^a)	1.1 (0.12 ^a)	0.29
<i>Fusarium oxysporum</i>	11.1 (0.38 ^a)	2.2 (0.23 ^a)	0.0 (0.00 ^a)	6.7 (0.41 ^a)	7.8 (0.45 ^a)	0.55
<i>Curvularia spp.</i>	41.1 (1.32 ^c)	3.3 (0.35 ^{ab})	30.0 (0.81 ^{bc})	3.3 (0.35 ^{ab})	0.0 (0.00 ^a)	0.61
<i>Phoma betae</i>	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	2.2 (0.15 ^a)	0.0 (0.00 ^a)	0.19
<i>Colletotrichum spp.</i>	0.0 (0.00 ^a)	2.2 (0.23 ^b)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.20
<i>Rhizoctonia solani</i>	2.2 (0.15 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.0 (0.00 ^a)	0.33

The mean separation indicates per pathogen the differences among the five farmers ($P \leq 0.05$).

Source: The two-way interactions between farmer and TLV species on percentage occurrence of seed-borne pathogens are seen in Annex 3 to Annex 12.

Colletotrichum spp. The lowest percentage occurrence of *Curvularia spp.* was detected for farmer 5, followed in order by farmers 3, 2, and 4 and highest for farmer 1. For *Colletotrichum spp.* the highest percentage occurrence of a single isolate was observed for farmer 2, and the remaining farmers were not significantly different from each other.

The difference between TLV species in the percentage occurrence of the *X. campestris* isolate (Annex 3) depends on the farmer, with farmer 1 showing occurrences very different from the other farmers. Similarly, a two-way ANOVA carried out on the *P. syringae* isolate (Annex 4) showed that the difference in occurrence between TLV species depended on the farmer, with *A. hybridus* and *B. pilosa* showing much more variation among farmers than *C. gynandra*. For the *Bacillus spp.* isolate (Annex 5), the results showed that the ranking of the species for each farmer was only different for Farmer 5. The results of a two-way ANOVA on the *A. alternata* isolate (Annex 6) depicted that the order of occurrence of the disease among the TLV species strongly depended on species and varied considerably.

Similarly, for the *Aspergillus spp.* isolate (Annex 7), the results showed that the difference between farmers depended on TLV species with farmer 4 showing the largest differences. For the two-way ANOVA carried out for the *F. oxysporum* isolate (Annex 8), the findings showed that the differences among, but also the ranking of the TLV species were different for each farmer. The results for the *Curvularia spp.* isolate (Annex 9) also showed that both the differences among and the ranking of the TLV species differed among farmers. For the *Phoma betae* isolate (Annex 10), the ranking of the TLV

species was consistent among the farmers, but the extent of the differences among the species differed among farmers.

A two-way ANOVA was also carried out to assess the interaction between farmer and TLV species on the *Colletotrichum spp.* isolate (Annex 11), demonstrating that the variation among TLV species was larger for farmer 2 than for the other farmers. For the *Rhizoctonia solani* isolate (Annex 12), Farmer 1 showed the largest difference among TLV species, especially because *A. hybridus* had a high value.

Pathogenicity tests

Table 6 depicts the occurrence of seed- and soil-borne pathogens on plants. The occurrence here refers to the presence of seed-borne pathogens on plant parts (showing typical symptoms of diseases) and were not scored or scaled. For pathogenicity tests of seed-borne diseases on plants, two bacterial and six fungal species were identified. The two bacterial species included *X. campestris* and *Bacillus spp.* while the fungal species were *A. alternata*, *F. oxysporum*, *Trichoderma spp.*, *Penicillium spp.*, *Erysiphe spp.* and *Pythium spp.* Several soil pathogens including *Bacillus spp.*, *Trichoderma spp.* and *Penicillium spp.* were also isolated from the plant parts. The farmers with an asterisk indicate that the observed pathogen were also present on seed.

Comparison of seed-borne pathogens on seed corresponds to pathogenicity on plants

The pathogenicity tests on the plants indicated that *F.*

Table 6. The occurrence of seed and soil-borne pathogens on plants.

Name of pathogen	Plant species		
	<i>Amaranthus hybridus</i>	<i>Cleome gynandra</i>	<i>Bidens pilosa</i>
Bacteria			
<i>Xanthomonas campestris</i>	Absent	Present (Farmer 2)*	Absent
<i>Bacillus</i> spp.	Absent	Present (Farmer 4)	Present (Farmer 2)
Fungi			
<i>Alternaria alternata</i>	Present (Farmer 1)*	Absent	Present (Farmer 3)*
<i>Fusarium oxysporum</i>	Present (Farmer 1)*	Present (Farmer 4)*	Present (Farmer 5)
<i>Penicillium</i> spp.	Present (Farmer 1)	Present (Farmer 2)	Present (Farmer 2)
<i>Trichoderma</i> spp.	Absent	Present (Farmer 4)	Absent
<i>Erysiphe</i> spp.	Absent	Absent	Present (Farmer 3)
<i>Pythium</i> spp.	Present (Farmer 4)	Absent	Absent

The words in parentheses represent the farmers; * observed pathogens were also present on seed.

oxysporum and *Penicillium* spp. had a wide range of infections as they were observed on all the three species, followed by *A. alternata* and *Bacillus* spp. as these pathogens were present on two species while *X. campestris*, *Trichoderma* spp., *Erysiphe* spp. and *Pythium* spp. had the least pathogenicity range, present only on one species. Occurrence of *X. campestris*, *A. alternata* and *F. oxysporum* on both seed and plants was observed. Occurrence of seed-borne pathogens on both the seed and plants was observed for farmers 1, 2, 3 and 4.

DISCUSSION

Seed health testing is important for four reasons: reduce seed-borne inoculum incidences and give an economic value to the seed lot, its requirements to satisfy the seed movement, elucidate seedling evaluation and causes of poor germination and give recommendation and perform seed lot treatment in order to eradicate seed-borne pathogens and reduce the risk of disease transmission (ISTA, 2015).

This study aims at identifying seed-borne pathogens in TLV seed from different farmers. This is primarily useful since the literature on seed-borne pathogens for TLV seed is scanty and farmers normally rely on literature for other crops like exotic cabbages which are relatively different in terms of production requirements or they rely on traditional production knowledge that is passed on through generations (Schipper, 2000). Unlike cabbage seed from relatively improved hybrid varieties, TLV seed predominantly is from traditional unimproved varieties. Therefore, in Zimbabwe, their production requirements are totally different from cabbage and TLV farmers should not rely on information on cabbage. Furthermore, this study investigated whether pathogens found on seed

showed pathogenicity to the plants. There is limited literature on pathogenicity tests for TLVs, hence this study aims at filling that gap.

Seed samples of three TLV plant species from five different farmers were compared for the percentage occurrence of seed-borne pathogen isolate on each seed. Furthermore, the study investigated whether pathogens found on seed show pathogenicity to the plants. Pathogenicity tests were conducted and showed pathogenicity effects on seeds and plants. Major pathogens included *X. campestris*, *A. alternata* and *F. oxysporum*. The seed-borne pathogens *X. campestris* and *Alternaria* spp. were also reported to be pathogenic to traditional leaf vegetables in several other studies (Sharma et al., 1980; Bradbury, 1986; Grubben and Denton, 2004), but no such reports were made on *F. oxysporum*. The results also indicated that seed sourced from farmers 1, 2, 3 and 4 had occurrence of seed-borne pathogens on both the seed and plants (Table 6). These results clearly show that farmers use bad seed.

Further, comparison on accumulative percentage occurrence for the 10 seed-borne pathogens observed (Table 5) showed wide ranges: farmer 3 (114.4), followed by farmer 1 (101.2), then, third, farmer 2 (91.0), the fourth, farmer 4 (68.9) and least, farmer 5 (54.51). The superiority of farmer 5 over other farmers was expected, because it is a research organization, which has much better knowledge on seed production compared to its counterparts. On the other hand, differences in the percentage occurrence of seed-borne pathogens among the sources may partially be explained by different crop management practices during seed development as previously reported by Bradbury (1986) and Naseri and Emami (2013). Bradbury (1986) reported that some TLVs are important hosts of *X. campestris* pv. *campestris*, and, therefore, likely sources of inoculum if diseased plants are left to grow and subsequently seed is harvested.

Farmers need seed of good quality to start with, for them to be able to satisfy the market requirements. The quality of the seed is comprised of many different attributes that add to the performance of the seed. Being free from seed-borne diseases, is figured out the most important one (Dube and Mujaju, 2013). Observations indicating that higher levels for percentage occurrence of seed-borne pathogen isolate on each seed by farmer (statistically significant at 5%) is also confirmed by the pathogenicity tests. Although, there are not yet established the quality standards of these plant species in literature, farmers require clean seed, that is free from seed-borne diseases for them to increase their yields and get better incomes from their crops.

Many seed-borne pathogens become active when seeds are sown, which may result in seed decay or pre- or post- emergence damping off (Agarwal and Sinclair, 1996), thereby reducing desired plant population in the farmers' fields. Moreover, many seed-borne pathogens, particularly, fungi induce qualitative changes in the physio-chemical properties of seeds such as protein content (Agarwal and Sinclair, 1996) that are fundamental in the germination process. For instance, Mathur and Sehgal (1964) reported that a collective of fungal pathogens contaminants, e.g. *Fusarium spp.*, reduced seed vigour and inhibited seed germination. Notably, some isolates of *Bacillus spp.* decreased seed germination or seedling growth (Broadbent et al., 1977).

The observation of the pathogens on both seed and plants and moderate to high virulence on tested plant species may explain their strong association with seed and ability to give rise to progressive disease development in the field. Such developments influence the ultimate crop yield and reduce the commercial value of the crop as well (Naseri and Mousavi, 2015). This may, therefore, give a positive indication of possible need of control measures. For example, in a study carried by Rude et al. (1999), infection of seed by *Alternaria spp.* greatly reduced seed germination of turnip. Although in this study we did not test the germination of seed, literature regarding these TLV seed has reported difficulties with seed germination (Cernansky, 2015) which cannot be overlooked the incidences of seed-borne as an important factor reducing the seed germination.

Virulence is the degree of pathogenicity exhibited by most of the pathogens and it is a measure that effectively differentiates pathogenic and non-pathogenic strains (Russell and Herward, 2005). All bacteria isolates gave a positive response on all three TLV plant species (Table 3) and with varied magnitudes, ranking as highly, moderately and weakly virulent pathogens based on host responses. Interestingly, among the tested plant species for virulence, none were found to possess a high level of resistance to the present bacteria pathogens observed in the study. Differential virulence on these TLV plant species are further confirmed by pathogenicity test. These results suggest, although the observed pathogens

have wide host range, these pathogens were sufficient to cause host-generated infections and if ignored may cause serious problems in TLV seed and vegetables production.

Erysiphe spp. was observed on *B. pilosa* during the late stage of growth, that is, seed development and seed maturation. *Erysiphe cruciferarum* in wild radish has been shown to affect the crop late and is destructive at the seed development stage (Djébali et al., 2009). Schippers (2000), Grubben and Denton (2004) also confirmed the presence of *Alternaria spp.* in East Africa causing damping off. The study results showed that the bacterium *X. campestris*, although present in high quantities on seed of all three plant species, exhibited pathogenicity on *C. gynandra* only and did not show up on *A. hybridus* and *B. pilosa* plants. *X. campestris* has already been reported as a serious pathogen, causing economic problems in vegetables production in Zimbabwe (Handiseni et al., 2008).

Considering its close association with *Cleome gynandra* seed, it poses a serious threat in *C. gynandra* production as well. On the same note, *Trichoderma spp.* was isolated from one sample of *C. gynandra* and it was detected in all the replicates. The plants had leaf spot. *Trichoderma spp.* can behave as an opportunistic pathogen. Harman et al. (2004) confirmed that particular strains of *Trichoderma spp.* in rare cases, are pathogenic to plants and have been reported to cause diseases of the crops such as apples, maize and alfalfa. Some *Trichoderma spp.* strains also grow on leaf surfaces. That probably we detected *Trichoderma spp.* in one of the study samples, may also pose a serious threat in *C. gynandra* production. Also, *Bacillus spp.* was isolated on all three plant species, *A. hybridus* seed and *C. gynandra* and *B. pilosa*. Some *Bacillus spp.* isolates can be beneficial, whereas others can be detrimental in crop production. Broadbent et al. (1977) reported that some *Bacillus spp.* increased plant growth by producing gibberellin or auxin or solubilizing phosphate, while others reduced germination.

CONCLUSION AND RECOMMENDATIONS

Although most of the pathogens observed in the study results have a broad host spectrum, pathogenicity and virulence tests substantiate that some pathogens were more likely to be closely associated with these traditional leafy vegetable seed species. All bacteria isolates gave a positive response on all three TLV plant species and with varied magnitudes, ranking as highly, moderately and weakly virulent pathogens based on host responses. However, none of the plant species were found to possess a high level of resistance to the present bacteria pathogens observed in the study. Furthermore, *X. campestris*, *F. oxysporum*, *A. alternata* and *Bacillus spp.* isolates were pathogenic to *A. hybridus*, *C. gynandra* and

B. pilosa plants as confirmed by pathogenicity tests. The occurrence of seed-borne pathogens on both the seed and plants for farmers 1, 2, 3 and 4 clearly showed bad seed. One further important area for future research is virulence testing for fungal pathogens using more samples collected from various places around Zimbabwe. Such tests will lead to better understanding of pathogens affecting TLV seed and production, and to more knowledge on certain pathogens prevalent in certain areas. There is limited literature reported on the economic losses caused by these pathogens on TLVs.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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ANNEX

Annex 1. Climatic data during crop growth of the origin sites of the seed.

Weather	Dec	Jan	Feb	March	April	May	June
Farmer 1							
Temperature (°C)	31	29	30	28	26	23	22
Rain (mm)	73	56	49	98	68.5	11.2	0.3
% cloud cover	24	36	27	33	25	17	5
% humidity	57	69	72	81	68	61	56
Bee activity	-	-	Mod	Mod	High	Low	-
Planting	Sole crop						
Farmer 2							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	-	Mod	High	Low	-
Planting	Intercropped with maize						
Farmer 3							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	100	49	46	85	29.5	17	0.8
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	Mod	Mod	High	Low	-
Planting	Intercropped with maize						
Farmer 4							
Temperature (°C)	31	29	30	28	26	22	21
Rain (mm)	110	57	63	29	15	10	1.5
% cloud cover	-	39	33	38	22	20	13
% humidity	-	67	76	79	69	67	63
Bee activity	-	-	Mod	Mod	high	-	-
Planting	Sole crop						
Farmer 5							
Temperature (°C)	29	27	28	27	-	-	-
Rain (mm)	105.7	14.5	16.7	24.8	-	-	-
% cloud cover	24	33	30	36	29	21	17
% humidity	57	68	67	77	70	69	64
Bee activity	-	-	-	-	-	-	-
Planting	Sole crop						

<https://www.worldweatheronline.com> Historical average weather 20/04/2017.

Annex 2. Geographical coordinates and climatic data during crop growth of the origin sites of the seed.

The seed was sourced from three different agro ecological regions of Zimbabwe: Mashonaland Central, Mashonaland West and Mashonaland East. The five seed sources were Horticultural Research Centre (HRC) (Farmer 5), Shamva (Farmer 4), Murehwa – two farmers (Macheya- Farmer 2 and Makondora- Farmer 3) and Chinhoyi (Farmer 1). HRC is a government institute located in Mashonaland East province and is under agro ecological region 2B (18° 11' 6.97" S: 31° 33' 6.95" E) at an elevation of 1688 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall for the area ranges from 850 to 900 mm. The mean annual temperature ranges from 18 to 29°C. During seed crop growth temperatures and rainfall ranged (December 2015 to March 2016) from 28.9 to 26.7°C and from 105.7 to 24.8 mm, respectively. Shamva is in Mashonaland Central province and located under agro ecological region 3 (17° 18' 56" S: 31° 34' 14" E) at an elevation of 953 m above sea level, receiving annual rainfall of 800 to 850 mm (Vincent and Thomas, 1960). The mean temperature for Shamva ranges between 21 to 31°C. During seed crop growth temperatures and rainfall ranged (December 2015 to June 2016) from 31 to 21°C and from 110 to 1.5 mm, respectively. Murehwa is located in Mashonaland East province under agro ecological region 3 (17° 38' 35.59" S: 31° 47' 2.40" E) at an elevation of 1400 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall ranges from 800 to 850 mm. The mean annual temperature ranges from 18 to 29°C. During seed crop growth temperatures and rainfall were (December 2015 to June 2016) 31 to 21°C and 100 to 0.8 mm, respectively. Chinhoyi is located in Mashonaland West province under agro ecological region 2B (17°22'00" S: 30°11'59" E) at an elevation of 1153 m above sea level (Vincent and Thomas, 1960). The mean annual rainfall and temperature ranges from 700 to 800 mm and 22 to 29°C, respectively. During seed crop growth temperatures and rainfall ranged from (December 2015 to June 2016) 31 to 22°C and 73 to 0.31 mm, respectively, (authors, <https://www.worldweatheronline.com> Historical average weather 20/04/2017), data presented as Annex 1. In Annex 3 to 12, the mean separation indicates per pathogen the differences among the interactions between farmers and three species (Duncan's multiple range least significant difference test, $P \leq 0.05$). For all cases, all values in parentheses are transformed values and mean separation.

Annex 3. The two-way interactions between the farmers and plant species percentage occurrence of *X. campestris*.

Farmer* species	Mean
Farmer 2 A. <i>hybridus</i>	0.0(0.0 ^a)
Farmer 3 A. <i>hybridus</i>	0.0(0.0 ^a)
Farmer 4 A. <i>hybridus</i>	0.0(0.0 ^a)
Farmer 5 A. <i>hybridus</i>	0.0(0.0 ^a)
Farmer 4 B. <i>pilosa</i>	0.0(0.0 ^a)
Farmer 5 B. <i>pilosa</i>	0.0(0.0 ^a)
Farmer 1 C. <i>gynandra</i>	0.0(0.0 ^a)
Farmer 1 A. <i>hybridus</i>	0.67(0.44 ^b)
Farmer 2 B. <i>pilosa</i>	9.33(1.97 ^c)
Farmer 5 C. <i>gynandra</i>	9.67(1.99 ^c)
Farmer 1 B. <i>pilosa</i>	10.0(2.00 ^c)
Farmer 2 C. <i>gynandra</i>	10.0(2.00 ^c)
Farmer 3 C. <i>gynandra</i>	10.0(2.00 ^c)
Farmer 3 B. <i>pilosa</i>	10.0(2.00 ^c)
Farmer 4 C. <i>gynandra</i>	10.0 (2.00 ^c)
LSD 5%	0.33

Annex 4. The two-way interactions between the farmers and plant species percentage occurrence of *P. syringae*.

Farmer*species	Mean
Farmer 2 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 1 <i>B. pilosa</i>	0.0(0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0(0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0(0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0(0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0(0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0(0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.67(0.44 ^{ab})
Farmer 1 <i>A. hybridus</i>	1.0 (0.50 ^{ab})
Farmer 4 <i>B. pilosa</i>	2.0 (0.99 ^{bc})
Farmer 5 <i>B. pilosa</i>	1.67 (1.23 ^c)
Farmer 3 <i>A. hybridus</i>	2.33 (1.33 ^c)
LSD 5%	0.63

Annex 5. The two-way interactions between the farmers and plant species percentage occurrence of *Bacillus* spp.

Farmer*species	Mean
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 1 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.33 (0.35 ^b)
LSD 5%	0.26

Annex 6. The two-way interactions between the farmers and plant species percentage occurrence of *A. alternate*.

Farmer*species	Mean
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0(0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.33(0.35 ^{ab})
Farmer 4 <i>B. pilosa</i>	0.33(0.35 ^{ab})
Farmer 1 <i>A. hybridus</i>	0.67 (0.69 ^{bc})
Farmer 3 <i>B. pilosa</i>	0.67 (0.69 ^{bc})
Farmer 1 <i>C. gynandra</i>	1.67 (1.23 ^{cd})
Farmer 5 <i>B. pilosa</i>	1.67 (1.23 ^{cd})
Farmer 3 <i>C. gynandra</i>	2.33 (1.33 ^d)
Farmer 2 <i>C. gynandra</i>	2.33 (1.33 ^d)
Farmer 4 <i>C. gynandra</i>	2.67 (1.38 ^d)
Farmer 2 <i>B. pilosa</i>	2.67 (1.44 ^d)
LSD 5%	0.57

Annex 7. The two-way interactions between the farmers and plant species percentage occurrence of *Aspergillus spp.*

Farmer*species	Mean
Farmer 5 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 1 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.33 (0.35 ^{ab})
Farmer 4 <i>B. pilosa</i>	2.0 (0.60 ^b)
LSD 5%	0.51

Annex 8. The two-way interactions between the farmers and plant species percentage occurrence of *F. oxysporum*.

Mean	Farmer*species
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.33 (0.35 ^{ab})
Farmer 2 <i>B. pilosa</i>	0.67 (0.69 ^{bc})
Farmer 4 <i>C. gynandra</i>	1.67 (0.88 ^{bc})
Farmer 1 <i>A. hybridus</i>	3.33 (1.14 ^c)
Farmer 5 <i>A. hybridus</i>	2.33 (1.34 ^c)
LSD 5%	0.67

Annex 9. The two-way interactions between the farmers and plant species percentage occurrence of *Curvularia* spp.

Farmer*species	Mean
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.33 (0.35 ^{ab})
Farmer 1 <i>A. hybridus</i>	0.67 (0.44 ^{ab})
Farmer 3 <i>B. pilosa</i>	1.67 (0.57 ^{ab})
Farmer 4 <i>A. hybridus</i>	0.67 (0.69 ^{ab})
Farmer 2 <i>B. pilosa</i>	1.0 (1.04 ^b)
Farmer 1 <i>C. gynandra</i>	5.0 (1.70 ^c)
Farmer 1 <i>B. pilosa</i>	6.67 (1.83 ^c)
Farmer 3 <i>C. gynandra</i>	7.33 (1.87 ^c)
LSD 5%	0.65

Annex 10. The two-way interactions between the farmers and plant species percentage occurrence of *P. betae*.

Farmer*species	Mean
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.67 (0.44 ^b)
LSD 5%	0.33

Annex 11. The two-way interactions between the farmers and plant species percentage occurrence of *Colletotrichum spp.*

Farmer*species	Mean
Farmer 1 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.67 (0.69 ^b)
LSD 5%	0.26

Annex 12. The two-way interactions between the farmers and plant species percentage occurrence of *R. solani*.

Farmer*species	Mean
Farmer 1 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 1 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 2 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 2 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 2 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 3 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 3 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 3 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 4 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 4 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 4 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 5 <i>A. hybridus</i>	0.0 (0.0 ^a)
Farmer 5 <i>B. pilosa</i>	0.0 (0.0 ^a)
Farmer 5 <i>C. gynandra</i>	0.0 (0.0 ^a)
Farmer 1 <i>A. hybridus</i>	0.67 (0.44 ^b)
LSD 5%	0.33