ASSESSMENT OF THREE SEED-BORNE BACTERIA ASSOCIATED WITH TOMATO (*SOLANUM LYCOPERSICUM L.*) SEEDS AFTER STORAGE AND THEIR EFFECT ON GERMINATION AND SEEDLINGS VIGOR

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SUMMARY

Poor seed quality is a significant factor affecting tomato productivity at the farm level. Tomato seeds were extracted from fruits of five tomato cultivars (Perfect pee, F_1-mongal, Dankukumi, UTC-18 and EKT-2) sourced from a conventional DelPHE-5 Research farm, Federal University of Agriculture, Abeokuta, Ogun State. *Xanthomonas campestris* pv. *vesicatoria* (Xcv), *Clavibacter michiganensis* subsp. *michiganensis* (Cmm)and *Pseudomonas syringae* pv. *tomato* (Pst) were isolated from tomato seeds after six months of storage in both 2015 and 2016. Pathogens were identified through colony morphology, biochemical and pathogenicity tests. Colony characteristics of Xcv and Cmm was determined on yeast dextrose carbonate (YDC) while Pst was inoculated on *Pseudomonas* F (PF) medium. *Pseudomonas syringae* pv. *tomato* recorded highest frequency of 56.10 and 61.36 % followed by *Xanthomonas campestris* pv. *vesicatoria* (31.71 and 22.73%) both in 2015 and 2016 respectively. The germination percentage was determined based on the number of normal seedlings emerged while vigor index was calculated as the product of germination percentage and total seedling length. At 7DAS, germination percentage ranged from 83.33 to 16.67% in both

years. At 14DAS, Perfect pee had the highest germination percentage (96.67%) in 2016 which was significantly different ($p \leq 0.05$) from the least value (16.67%) recoded in EKT-2 in 2015. Perfect pee had highest significant vigor index of 1,002.70 compared with other cultivars. Results showed that contaminated tomato seeds could be a primary source of inoculum. Therefore, seed treatment would be of high benefit to reduce disease incidence on the field.

Keywords: Bacteria, cultivars, germination, seed-borne, tomato, vigor index.

In Nigeria, production of tomato is majorly done by resource limited small-scale farmers who use stored tomato seeds of unknown quality extracted from previous crop to raise the next crop. The seeds of unknown quality contribute to reduced yields at harvest (1) and serve as route for disease transmission (10, 9,7). Seed-borne pathogenic bacteria act as primary inoculum source for many important vegetable diseases. Among others are bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*), bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) and bacterial speck (*Pseudomonas syringae* pv. *tomato*). When conditions are optimal for these bacterial diseases, losses in marketable yield can be up to 60% in some fields (6). Damage from these diseases may range from a light spotting of the foliage to almost complete defoliation of the plant, with corresponding impacts on photosynthesis and production potential. When present, fruit lesions disfigure and reduce the marketability of fresh-market fruits. These bacterial diseases are economically important to their respective hosts and in most cases, infested seeds and seedlings serve as a primary inoculum source for epidemics in the greenhouse and in the field (3). Seeds may even be responsible for the introduction of exotic diseases to new areas or sometimes may also account for re-emergence of past diseases (3). Seed health testing and seed treatments are regularly used to exclude seed-borne bacterial inoculum (3). Tomatoes grown from locally produced seeds have been reported to harbor unique populations of *Xanthomonas* (2; 14). *Clavibacter michiganensis* subsp. *michiganensis* and *P. syringae* pv. *tomato* also affect can effectively transmit through contaminated seeds (17, 8).

On leaves of tomato or *Capsicum*, lesions appear as irregular water-soaked areas, at first green, later becoming brown and necrotic when *Xanthomonas campestris* pv.

vesicatoria infects. Initial leaf symptoms are small, circular-to-irregular, dark lesions, which may be surrounded by a yellow halo. The lesions tend to concentrate on the leaf edges and tip and may increase in size to a diameter of 3-5 mm. Infected leaves may develop a scorched appearance (6). When spots are numerous, foliage turns yellow and eventually dies, leading to defoliation of the lower portion of the plant. Typical symptoms caused by *Clavibacter michiganensis* subsp. *michiganensis* are canker formation at the site of inoculation, yellowing and marginal necrosis and/or wilting of true leaves. Tomato foliage infected with the canker organism has distinctive black leaf edges with no spotting on the interior of the leaves. Sometimes a thin yellow border is present between the dead leaf margins and healthy tissue (6). Speck lesions (*P. syringae* pv. *tomato*) look similar initially but are surrounded by a more distinct yellow halo; lesions are often in streaks and the yellow haloes run together to give large chlorotic areas (4). Bacterial speck lesions may occur anywhere on the foliage, stems or fruit. Symptoms are very difficult to visually distinguish from bacterial spot and can be confused with young, early blight lesions. On leaves, symptoms appear as black specks, usually no more than 2 mm in diameter, which are usually surrounded by a yellow halo (6). Speck lesions sometimes cause distortion of the leaf, as the infection restricts the expansion of leaf tissue. Lesions are often concentrated near leaf edges, and in some cases, leaf margins burn, resembling bacterial canker. When numerous, lesions may coalesce, and entire leaflets may die. Severely infected seedlings may become stunted.

Pathogen-free seeds are one of the important factors for good plant population and harvest (5). Health of seeds can be affected by direct infection by pathogens or through contamination (15). Infected seeds by a pathogenic organism may not even germinate, causing infection to seedlings and growing plants (5). As asserted by (5) that planting high quality seeds could contribute to increase germination as well as vegetable yield. This study therefore was out to assess the presence of *Xanthomonas campestris* pv. *vesicatoria*, *Clavibacter michiganensis* subsp. *michiganensis* and *Pseudomonas syringae* pv. *tomato* in five tomato seeds cultivars after storage for six months and to evaluate their effect on seed germination and seedlings vigor index.

MATERIALS AND METHODS

Experimental site

The experiment was conducted in the Plant Tissue Culture Laboratory of the Department of Crop Protection, College of Plant Science and Crop Production, Federal University of Agriculture Abeokuta (FUNAAB) in the year 2015 and 2016.

Source of seeds and preservation

Freshly harvested five tomato fruits (cvs. Perfect Pee, F1-Mongal, Dankukumi, UTC-18 and EKT-2) were sourced from a conventionally established tomato plot in DelPHE-5 Research farm, FUNAAB, Ogun State. The extraction of seeds was done by hands in order to minimize mechanical damage. The seeds of each variety were air-dried to 12% moisture content at room temperature (30±2^o C) and relative humidity between 75 and 80%. Five grams seeds of each variety were placed separately into clothes made of cotton materials, tied and kept in a seal drum at room temperature in Tissue Culture Laboratory, Department of Crop Protection, FUNAAB. Seeds were preserved for six months (November-April) in both 2015 and 2016 respectively.

Isolation and identification of pathogens

The seeds were surface sterilized with 1% sodium hypochlorite (NaOCl) for 5 min, rinsed in three changes of sterile distilled water and air-dried on sterile filter paper. Fifty randomly selected seeds samples were aseptically placed inside Petri dish contained nutrient agar (15.00 g/L agar, 5.00 g/L peptone, 5.00 g/L NaCl, 2.00 g/L yeast extract, 1.00 g/L beef extract). Plates were incubated at 28±2^{o}C for 48 hrs. Isolates were purified by sub-culturing before identities were confirmed by morphological and biochemical tests. To confirm pathogen identification, one distinct representative colony of each suspected bacterial genus was picked from each plate and streaked on different Petri plates containing yeast dextrose carbonate (YDC) medium for *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* while *Pseudomonas* F (PF) medium for *Pseudomonas syringae* pv. *tomato.* Four biochemical tests were conducted, which included Arginine dihydrolase, Kovac's oxidase test, gram reaction (KOH solubility test) and nitrate reduction according to the procedures described by (11), (18) and (15).

Pathogenicity test

Pathogenicity of the bacterial isolates was determined by spraying 1x 10^8 CFU/ml of each inoculum's suspension into the leaves of 4-week old tomato plants (cv. Roma round). Tomato plants were sprayed with the bacterial suspensions using a hand-held sprayer for each inoculum suspension until run-off. Control plants were sprayed with sterile distilled water. After inoculation, plants were covered with plastic bags for 48 hours in order to obtain high humidity and kept under screenhouse conditions at 26±2°C, relative humidity between 85 and 90%. Symptoms were monitored daily.

Soil assay for germination percentage and seedling vigor

Thirty randomly selected seeds were sown in nursery trays containing steam-sterilized sandy loam soil, with five replicates per tomato cultivar, in a screenhouse. Normal seedlings that emerged from soil were counted at 7 and 14 days to determine germination percentage. Light spray irrigation was applied when necessary using hand held knapsack sprayer. At 14th day after sowing, trays containing the seedlings were flooded with water to loosen the soil and the soil washed away from the roots. Shoot and root lengths were measured from the soil surface to the tips of shoot and root, respectively. Total seedling length was taken as addition of shoot and root lengths. The vigor index was calculated using the following formula.

Vigor index (VI) = germination percentage x total seedling length (cm) (13; 16) where total seedling length = mean root length (cm) + mean shoot length (cm). Shoot and root lengths were each measured from the soil surface.

Data analysis

All data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS), version 9.1, 1990 and mean separation by Duncan's Multiple Range Test at 5% level of probability ($p \leq 0.05$).

RESULTS

Pathogens and their phenotypic characteristics

On yeast dextrose carbonate (YDC) agar, colonies were large, smooth-domed, mucoid-fluidal and yellow with entire edges. This revealed the morphological characteristics of *Xanthomonas campestris* pv. *vesicatoria* while yellow, domed and mucoid colony appearance showed the characteristics of *Clavibacter michiganensis* subsp. *michiganensis.* After 2 days colonies of *Pseudomonas syringae* pv. *tomato* appeared as creamy, white, round, entire and flat on *Pseudomonas* F (PF) medium. The biochemical tests confirmed the presence of *Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. Only *Clavibacter michiganensis* subsp. *michiganensis* was gram positive but *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria* were gram negative.

Pathogenicity test

All isolated strains caused typical disease symptoms on tomato leaves after 72 hours of inoculation. Plants inoculated with sterile distilled water produced no symptoms. Small, circular-to-irregular, dark lesions (2-3 mm diameter), surrounded by a yellow halo were observed with *Xanthomonas campestris* pv. *vesicatoria* inoculation. Leaves sprayed with *Clavibacter michiganensis* subsp. *michiganensis* formed canker at the site of inoculation with yellowing and marginal necrosis at the leaf's edges. Black specks (about 2 mm diameter) were observed on leaves inoculated with *Pseudomonas syringae* pv. *tomato* surrounded by a yellow halo. Bacteria were re-isolated on yeast dextrose carbonate (YDC) medium and *Pseudomonas* F (PF) medium accordingly from symptomatic leaves and Koch's postulates were confirmed.

Frequency of seed-borne bacteria isolated from stored tomato seeds

Table 1 shows that three different bacteria were isolated from stored tomato seeds in both 2015 and 2016. Isolates were identified as *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis*. The most prevalent was *Pseudomonas syringae* pv. *tomato* which accounted for 56.10 and 61.36% occurrence in both 2015 and 2016 respectively (Table 2). Thereafter, *Xanthomonas campestris* pv. *vesicatoria* had 31.71% and 22.73% and while *Clavibacter michiganensis* subsp. *michiganensis* recorded 12.19% and 15.91% in both 2015 and 2016 respectively (Table 2).

Germination percentage and seedling vigor

Table 3 shows the results of germination test conducted on the five cultivars of stored tomato seeds at 7 and 14 days after sowing (DAS). At 7DAS, germination percentage ranged from 83.33 to 16.67% while at 14DAS percentage germination ranged from 96.67 to 30.00% in both years. In 2016, Perfect pee recorded highest germination percentage of 83.33 and 96.67% at 7 and 14 days after sowing (DAS)respectively, and were significantly different ($p \leq 0.05$) from the germination percentage of all other cultivars. Cultivar EKT-2 in 2015 had the lowest germination percentage of 16.67 and 30.00% in 7 and 14 DAS respectively. Vigor index varied significantly ($p \leq 0.05$) among cultivars (Table 3). Perfect Pee had highest vigor index of 1002.70 in 2016 and was significantly different ($p \leq 0.05$) from lowest vigor index (453.70) recorded in EKT-2 in the same year. Dankukumi in 2016 and UTC-18 had 962.00 and 910.30 vigor indexes respectively but were not significantly different ($p \geq 0.05$) from each other.

Table 1: Isolated bacteria strains from stored tomato seeds in 2015 and 2016

Cultivar	Year	Bacteria Genus
Perfect Pee	2015	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*
	2016	*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato*
F_1-mongal	2015	*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato*, *Clavibacter michiganensis* subsp. *michiganensis*
	2016	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*
Dankukumi	2015	*Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato*
	2016	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*
UTC-18	2015	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*
	2016	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria, Clavibacter michiganensis* subsp. *michiganensis*
EKT-2	2015	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*
	2016	*Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria*

Table 2: Frequency of seed-borne bacterial strains isolated from stored tomato seeds

Bacterial species	Number of isolates		% Frequency	
	2015	2016	2015	2016
Pseudomonas syringae pv. *tomato*	23	27	56.10	61.36
Xanthomonas campestris pv. *vesicatoria*	13	10	31.71	22.73
Clavibacter michiganensis subsp. *michiganensis*	5	7	12.19	15.91
Total	41	44	100	100

Table 3: Germination (%) and vigor index of stored tomato seeds at 7 and 14 days after sowing

Cultivar	Year	Germination (%)		Vigor Index
		7DAS	14DAS	14DAS†
Perfect pee	2015	40.00^{cd}	23.33^{e}	251.70^{d}
	2016	83.33^{a}	96.67^{a}	1002.70^{a}
F_1-mongal	2015	40.00^{cd}	46.67^{cde}	293.70^{d}
	2016	60.00^{abc}	70.00^{bc}	668.70^{bc}
Dankukumi	2015	40.00^{cd}	46.67^{cde}	411.70^{cd}
	2016	70.00^{ab}	76.67^{ab}	962.00^{ab}
UTC-18	2015	43.33^{c}	46.67^{cde}	398.70^{cd}
	2016	60.00^{abc}	73.33^{ab}	910.30^{ab}
EKT-2	2015	16.67^{d}	30.00de	220.00^{d}
	2016	50.00^{bc}	53.33^{bcd}	453.70^{cd}

Values with the same superscript within each column are not significantly different by DMRT at ($p \leq 0.05$), †DAS: Days after sowing

DISCUSSION

The study provided additional knowledge on the identity of bacterial pathogens associated with stored tomato seeds. This gives insight on the management strategies to reduce the impact of seed-borne diseases and help in ensuring bumper harvest. The results of conventional identification of strains using bacteriological methods indicated that *Pseudomonas syringae* pv. *tomato, Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* were associated with tomato seeds. The investigation showed a high level of pathogenicity, inducing symptoms similar to those in natural infection of tomato plants seven days after spray-inoculation. Shila (18) detected *Pseudomonas* in the seeds of eight cucurbits that KOH solubility test and gram reaction showed that the bacterium was gram negative. In this study, *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* were isolated in tomato seeds and majority of which belonged to *Pseudomonas syringae* pv. *tomato*. This showed that seeds act as primary inoculums source for many important vegetable crops. *Clavibacter michiganensis* subsp. *michiganensis* was detected in tomato seeds (19). The results of the study indicated that *Pseudomonas syringae* pv. *tomato* had the highest frequency. These bacteria diseases are economically important to their respective hosts and in most cases, infested seeds and seedlings serve as a primary inoculums source for epidemics in the greenhouse and in the field (3).

CONCLUSION

The findings of the present study give an indication of the presence of *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* in tomato seeds. The bacteria isolated and identified as *Pseudomonas syringae* pv. *tomato*, *Xanthomonas campestris* pv. *vesicatoria* and *Clavibacter michiganensis* subsp. *michiganensis* were associated with tomato seeds and were pathogenic. Therefore, tomato seeds should be subjected to health test for bacteria before sowing. Further studies need to be undertaken in order to get the detailed information pertaining to the identities of the bacteria with a view to developing pathogen targeted and sustainable management approaches.

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