EFFECT OF THREE STERILIZING AGENTS ON SEED VIABILITY, SEEDLING VIGOR AND OCCURRENCE OF SEED-BORNE BACTERIAL PATHOGENS OF TWO TOMATO CULTIVARS

[*1]Ganiyu S. A., [2]Popoola A. R., [2]Imonmion J. E., [2]Uzoemeka I. P. and [2]Ojo K. O.
[1]Department of Agronomy, Federal University, Kashere, P.M.B. 0182, Gombe State, Nigeria
[2]Department of Crop Protection, Federal University of Agriculture, Abeokuta, P.M.B. 2240, Ogun State, Nigeria
*Author for correspondence: ganiyu.sikiru@yahoo.com; +2348136392050

SUMMARY

Efficient seeds surface sterilization and germination is a precondition for successful regeneration and transformation of tomato. Seeds from two tomato cultivars (UTC-18 and F_1-mongal) were used in this study. The seeds were treated with sterilizing agents, which included ethanol (70% v/v), hydrogen peroxide (3% v/v) and sodium hypochlorite (2% v/v). Treated and control seeds (30 seeds per plate) were placed on Nutrient agar (NA) amended with 2-3 drops of Benlate (0.5% w/v). The occurrence of Xanthomonas campestris *pv. vesicatoria, Pseudomonas syringae pv. tomato and Clavibacter michiganensis subsp. michiganensis gave a good indication of the results. At seventh day after sowing, percent germination was 96.67% for UTC-18 followed by 95.30% for F_1-mongal, when ethanol was used for surface sterilization and these values were not significantly different ($p \leq 0.05$) from 93.30% in F_1-mongal when NaOCl was used as sterilizing agent. At 14th day after sowing, the highest percentage seeds germination of 98.00% was recorded for ethanol-F_1-mongal treated seeds, which was not significantly different from 97.72% in NaOCl-F_1-mongal treated, 97.00% in ethanol-UTC-18 treated and 96.67% in NaOCl-UTC-18 treated seed lots. Application of sterilizing agents significantly reduced microbial loads, improved seedlings quality and vigor. Thus, the use of ethanol at 70% and NaOCl at 2% for 5 min would be suitable as tomato seeds surface sterilizing agents to reduce microbial loads, which would produce healthy seedlings for providing cotyledon and hypocotyls explants for use in tissue culture.*

Keywords: Germination, sterilizing agents, tissue culture, tomato, seedling vigor.

TOMATO is one of the most important fruits vegetables worldwide. Major traits targeted for genetic modification include yield, enhanced tolerance to biotic and abiotic stresses. Seeds and different parts of tomato seedlings have been used as explants (Kumria *et al*., 2003; Popoola *et al*., 2015). Along with many other factors that contribute to poor yield of tomato are seed-borne pathogens, as this affects seed viability and germinability. Over 50 microorganisms are reported to be seed-borne in nature in different vegetable seeds (Richardson, 1990). These pathogens induce seed deterioration and serve as source of primary inocula of many crop diseases in both nursery and field as expressed by (Gitaitis and Walcott, 2007). However, production of a high number of contaminant-free seedlings from a limited seed supply harvested from open field remains a challenge (Barampuram *et al*., 2014). Seeds from open field are often contaminated with pathogens such as fungi and bacteria (Ahmad *et al*., 2012; Ganiyu *et al*., 2018). Ethanol, sodium hypochlorite and calcium hypochlorite are the most often used agents for sterilization of plant material including seeds (Barampuram *et al*., 2014). Chlorine gas and hydrogen peroxide have been successfully used for sterilization of Arabidopsis and soybean seeds (Di *et al*., 1996) and other seeds and plant materials (Amjad *et al*., 2004; Cavusoglu and Kabar, 2010).

Seed germination might be problematic because of microbial contamination (Wang *et al*., 2011). To enable high tomato seed germination rate and production of high-quality sterile seedlings, this study, therefore, was out to determine the occurrence of seed-borne pathogenic bacteria, the seed viability and seedlings vigor of two tomato cultivars as influenced by sodium hypochlorite, hydrogen peroxide and ethanol as sterilizing agents.

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).

Source of seeds and soil sterilization

Seeds of two tomato (UTC-18 and F_1-Mongal) cultivars were obtained from the Plant Tissue Culture Laboratory, Federal University of Agriculture, Abeokuta (FUNAAB)**.** Sandy-loam soil was steam-sterilized at 120°C for 30 minutes. The sterilized soil was kept inside polythene bags for one week before use.

Treatments, experimental design and seeds sterilization

Treatment consisted of two tomato seeds cultivars (UTC-18 and F_1-Mongal) and three sterilizing agents (NaOCl solution (2%v/v), ethanol (70% v/v) and hydrogen peroxide (3% v/v)). Tomato seeds were sorted by hand to select for healthy (not wrinkled) and deformed (wrinkled) seeds. These were surface sterilized with sodium hypochlorite (NaOCl), ethanol (C_2H_5OH) and hydrogen peroxide (H_2O_2) for 5 minutes, rinsed in three changes of sterile distilled water and air-dried in a laminar flow hood. Seeds treated with sterile distilled water served as control. The experiment was laid out in a completely randomized design with three replications. Treated and control seeds (30 seeds per plate) were placed on Nutrient agar (NA) amended with 2-3 drops of benlate (0.5% w/v).

Pathogen isolation, identification and pathogenicity test

Seeds colonized by bacterial pathogens were monitored for 10 days. Bacteria were isolated, identified and pathogenicity tests carried out according to the method employed by Ganiyu *et al*. (2018). Percentage frequency of occurrence was estimated using the formula below:

$$\text{Frequency (\%)} = \frac{Number\ of\ seeds\ colonized\ by\ pathogens}{Total\ number\ of\ seeds\ plated}\ x\ 100$$

Germination test and seedlings vigor

Thirty randomly selected seeds were sown in nursery trays contained steam-sterilized sandy- loam soil with five replicates per tomato cultivar in Screen house. Healthy seedlings were counted after 7 and 14 days of sowing to determine germination percentage. Number of seedlings that emerged from healthy and deformed seeds, as well as dead (non-geminated) seeds were recorded.

$$\text{Germination percentage} = \frac{Number\ of\ seedlings\ emerged}{Number\ of\ seeds\ planted} \times 100$$

The same seedlings were used to determine vigor index at 14 days after sowing. Light irrigation was applied when necessary, with the aid of hand-held knapsack sprayer. At the 14th day after sowing, the trays containing the seedlings were flooded with water to loosen the soil and the soil was washed off the roots. Shoot and root lengths were measured with meter rule 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 test involved measurements of root and shoot lengths of seedlings and the percentage seedling emergence. Vigor index was calculated by multiplying the total seedling length and germination percentage and expressed as number, where seedling length total was calculated as sum of mean root and mean shoot length in centimetres.

Data analysis

Data collected were subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) package, version 9.1 (2003) and differences among means of the treatments were separated by Duncan Multiple Range Test at ($p \leq 0.05$).

RESULTS

Table 1 shows the effects of sterilizing agents on the occurrence of seed-borne bacterial pathogens of tomato. Treatments showed variations in their performance in both varieties on the occurrence of *Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas syringae* pv. *tomato* and *Clavibacter michiganensis* subsp. *michiganensis*. Sodium hypochlorite (NaOCl) suppressed the microbial load of *Xanthomonas campestris* pv. *vesicatoria* to 13.20% and 15.25% in both UTC-18 and F_1-Mongal cultivars, respectively. These values were not significantly different ($p \geq 0.05$) from 21.10% and 18.50% but significantly lower ($p \leq 0.05$) than 53.55% and 70.85% obtained from UTC-18 and F_1-Mongal, respectively. Occurrence of *Pseudomonas syringae* pv. in UTC -18 ranged from 24.05 to 84.75% while the frequency in F_1-Mongal ranged from 14.95 to 94.65%. When UTC-18 and F_1-Mongal were treated with ethanol (10.70% UTC-18; 4.85% F_1-mongal), the incidence of *Clavibacter michiganensis* subsp. *michiganensis* was significantly reduced ($p \leq 0.05$) compared with H_2O_2 (38.60% UTC-18; 23.30% F_1-Mongal) and untreated control (55.50% UTC-18; 61.75% F_1-Mongal).

Table 1: Effect of surface sterilization with ethanol, H_2O_2 and NaOCl on percentage frequency of occurrence of seed-borne pathogenic bacteria on two tomato cultivars.

Cultivar	Treatment	*Xanthomonas campestris* pv. *vesicatoria*	*Pseudomonas syringae* pv. *tomato*	*Clavibacter michiganensis* subsp. *michiganensis*
UTC -18	Control	87.60^{a}	84.75^{a}	55.50^{a}
	Ethanol	21.10^{d}	24.05^{d}	10.70^{d}
	H_2O_2	53.55^{c}	63.85^{c}	38.60^{b}
	NaOCl	13.20^{d}	27.15^{d}	19.65^{c}
F_1-Mongal	Control	99.75^{a}	94.65^{a}	61.75^{a}
	Ethanol	18.50^{d}	14.95^{de}	4.85^{e}
	H_2O_2	70.85^{b}	75.05^{ab}	23.30^{c}
	NaOCl	15.25^{d}	16.15^{e}	5.05^{e}

Values with the same letter within each column are not significantly different by DMRT at $p \leq 0.05$.

On the seventh day after sowing, percentage emergence of healthy and deformed seedlings was recorded (Table 2). Percent emergence (healthy seedlings) was the highest in UTC-18 (96.67%)

followed by 95.30% recorded for F_1-Mongal when ethanol was used to sterilize the seeds but was not significantly different ($p \geq 0.05$) from 93.30% in F_1-Mongal when NaOCl was used as sterilizer. When hydrogen peroxide (H_2O_2) was used as treatment, the percentage emergence of healthy seedlings was significantly low ($p \leq 0.05$) in UTC-18 (50.00%) and F_1-Mongal (56.67%) compared with 96.67 and 95.30 % (ethanol), 90.00 and 93.30 % (NaOCl) in UTC-18 and F_1-Mongal, respectively. There were significant differences ($p \leq 0.05$) observed in percentage emergence of seedlings from healthy seeds which ranged from 1.00 to 21.67 %. F_1-Mongal had the highest significant deformed seedlings of 21.67 % when sterile distilled water was used.

Table 2: Effect of surface sterilization on tomato seedling emergence seven days after sowing

Cultivar	Treatment	Percentage seedling emergence	
		Healthy	Deformed
UTC -18	Control	53.30^{b}	21.20^{a}
	Ethanol	96.67^{a}	1.00^{bc}
	H_2O_2	50.00^{b}	20.70^{a}
	NaOCl	90.00^{a}	2.20^{b}
F_1-Momgal	Control	53.33^{b}	21.67^{a}
	Ethanol	95.30^{a}	1.70^{bc}
	H_2O_2	56.67^{b}	21.25^{a}
	NaOCl	93.30^{a}	3.00^{b}

Values with the same superscript within each column are not significantly different by DMRT at $p \leq 0.05$

Parameters measured on the 14th day after sowing are shown in Table 3. The highest percentage of healthy seedlings (98.0 %) was recorded in ethanol-F_1-Mongal treated seeds, which was not significantly different from 97.72 % in NaOCl-F_1-Mongal treated, 97.0 % in ethanol-UTC-18 treated and 96.67 % in NaOCl-UTC-18 treated seed lots. Percentage germination of seeds (healthy seedlings) ranged from 0.5 to 24.83 %. Application of sterilizing agents improved the seedling vigor. NaOCl-UTC-18 treated seeds had the highest vigor index of 1,101.43 which was not significantly different from 1,099.33 in NaOCl-F_1-Mongal treated seed lots.

Table 3: Effect of surface sterilization on seed emergence and seedling vigor index 14 days after sowing.

Cultivar	Treatment	Percentage seedling emergence		Seedling vigour
		Healthy	Deformed	
UTC-18	Control	56.67^{c}	21.03^{a}	780.67^{c}
	Ethanol	97.00^{a}	0.50^{b}	992.87^{b}
	H_2O_2	50.67^{bc}	24.83^{a}	944.66^{b}
	NaOCl	96.67^{a}	1.33^{b}	$1{,}101.43^{a}$
F_1-momgal	Control	56.67^{b}	21.23^{a}	891.87^{c}
	Ethanol	98.00^{a}	0.95^{b}	990.33^{b}
	H_2O_2	56.67^{b}	20.50^{a}	790.46^{c}
	NaOCl	97.72^{a}	1.28^{b}	$1{,}099.33^{a}$

Values with the same superscript within each column are not significantly different by DMRT at $p \leq 0.05$

DISCUSSION

Among the agricultural inputs, seed is the most important one for crop production (Hamim *et al*., 2014) and this study showed that tomato seeds used were infected with seed-borne pathogens. Study conducted by Ganiyu *et al*. (2018) on the assessment of seed-borne bacteria associated with tomato seeds showed that UTC-18 and F_1-momgal were contaminated with bacterial pathogens, which agreed with this study. Barampuram *et al*. (2014) opined that it would be a challenge when heavily infected seeds are germinated *in-vitro*. F_1-mongal was more contaminated by seed-borne bacterial pathogens compared with UTC-18. In all the treatments applied, germination percentage of the untreated control seed lots was low. Hamim *et al*. (2014) asserted that there was a highly positive relationship between germination failure and prevalence of seed-borne pathogen infections.

The use of H_2O_2 resulted in poor germination, thus it was not effective as sterilizing agent for tomato seeds. Poor germination in tomato seeds might be because of negative effect of H_2O_2 on seeds as well as pathogens infection. Dolatabadian and Modarressanavy (2008) reported that 5% concentration of H_2O_2 negatively affected seeds germination in sunflower and rape seeds. Ethanol and NaOCl were effective in suppressing seed-borne pathogens, improved seeds germination and seedlings vigor index. Similarly, Baiyeri and Mbah (2006) used sodium hypochlorite for surface sterilization of *Treculia africana* and reported an increased seed germination rate. Sodium hypochlorite used in laboratory by Barampuram *et al*. (2014) and brought about good quality of cotton seeds with low or medium levels of contamination.

When H_2O_2 was used as sterilizing agent, the development of most of the germinated seeds was arrested as the cotyledon failed to unfold and hypocotyls failed to elongate with many seeds' dead, which could not be used for cotyledons and hypocotyls explants. Surface sterilization of rice seeds was ineffective when hydrogen peroxide was used (Miche and Balandreau, 2001). The results of this study did not agree with the previous studies that hydrogen peroxide treatment was effective for surface sterilization and improved germination rates for both pine (Hoefnagels and Linderman, 1999; Cram and Fraedrich, 2009), safflower seeds (Lizarraga-Paulin *et al*., 2013) and tomato seeds (Molan *et al*., 2010). Improved seedlings vigor recorded in both F_1-Mongal and UTC-18 seeds treated with ethanol (70% v/v) and NaOCl (2% v/v) for 5 minutes, depicted that they had eliminated inoculum from the seeds. Fatmi *et al*. (1991) stated that the use of pathogen-free seeds, obtained naturally or by chemical treatments, would eliminate a potential source of inoculum. It also showed that they were not phytotoxic to the treated seeds and so were efficient at these concentrations and short duration. Sen *et al*. (2013) also observed that NaOCl improved growth and development of *Achyranthes aspera*, using seeds as explant, at lower concentration and short duration and that plant materials should be exposed for a short period of time when ethanol is used. In this study, we found out that ethanol performed best as sterilizing agent with the lowest contamination of seed-borne bacterial pathogens and highest germination rates followed by NaOCl.

CONCLUSION

It could be concluded that the use of ethanol at 70 % and NaOCl at 2 % as surface sterilizing agents for 5 minutes would be suitable to reduce microbial load, achieve better germination and seedling vigor index for tomato seeds that would produce healthy seedlings for cotyledon and hypocotyl

explants in tissue culture. Also, if the use of ethanol at 70 % and NaOCl at 2 % is employed by tomato farmers before sowing, and with good farm management practices, it could reduce the effects of *Xanthomonas campestris* pv. *vesicatoria* (spot), *Pseudomonas syringae* pv. *tomato* (speck) and *Clavibacter michiganensis* subsp. *michiganensis* (canker) on tomato resulting in better germination rates and vigor, which would translate to higher fruit yields. However, ethanol would be a better choice sterilizing agent at 70 % for 5 minutes over NaOCl.

REFERENCES

Ahmad, F., Daglish, G. J., Ridley, A.W. and Walter, G. H. 2012. Responses of *Tribolium castaneum* to olfactory cues from cotton seeds, the fungi associated with cotton seeds, and cereals. *Entomol. Exp. Appl*.,145:272–281

Amjad, H., Shafqat, F., Nayyer, I. and Rubina, A. 2004. Influence of exogenous application of hydrogen peroxide on root and seedling growth on wheat (*Triticum aestivum* L.). *Int. J. Agric. Biol. Eng.,* 6:366–369

Baiyeri, K. P. and Mbah, B. N. 2006. Surface sterilization and duration of seed storage influenced emergence and seedling quality of African breadfruit (*Treculia africana* Decne). *African Journal of Biotechnology*, 5:1393–1396

Barampuram, S., Allen, G. and Krasnyanski, S. 2014. Effect of various sterilization procedures on the in vitro germination of cotton seeds. *Plant Cell Tissue and Organ Culture*, 118(1):179-185 DOI 10.1007/s11240-014-0472-x

Cavusoglu, K. and Kabar, K. 2010. Effects of hydrogen peroxide on the germination and early seedling growth of barley under NaCl and high temperature stresses. *EurAsian J. BioSci.,* 4:70–79

Cram, M. M. and Fraedrich, S. W. 2009. Seed diseases and seedborne pathogens of North America. Tree Planters' Notes 53(2):35–44

Di. R., Purcell, V., Collins, G. B. and Ghabrial, S. A. 1996. Production of transgenic soybean lines expressing the bean pod mottle virus coat protein precursor gene. Plant Cell Rep. 15:746–750

Dolatabadian, A. and Modarressanavy, S. A. M. 2008. Effect of ascorbic acid, pyridoxine and hydrogen peroxide treatments on germination, catalase activity, protein and malondialdehyde content of three oil seeds. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 36:61–66

Fatmi, M., Schaad, N. W. and Bolkan, H. A. 1991. Seed treatments for eradicating and *Clavibacter michiganensis* subsp. *michiganensis* from naturally infected tomato seeds. *Plant Disease*, 75: 383-385

Ganiyu, S.A., Popoola, A. R., Agbolade, J.O., Imonmion, J.E., Uzoemeka, I. P. and Akinola, O. C. 2018. Assessment of three seed-borne bacteria associated with tomato (*Solanum lycopersicum* L.) seeds after storage and their effect on germination and seedlings vigor. *Nigerian Journal of Plant Protection,* 32(1): 49-60.

Gitaitis, R. D. and Walcott, R. R. 2007. The epidemiology and management of seed-borne bacterial diseases. *Annual Review of Phytopathology*, l45: 371–397

Hamim, I., Mohanto, D. C., Sarker, M. A. and Ali, M. A. 2014. Effect of seed borne pathogens on germination of some vegetable seeds. *Journal of Phytopathology and Pest Management*, 1(1): 34-51

Hoefnagels, M. H. and Linderman, R. G. 1999. Biological suppression of seedborne *Fusarium* spp. during cold stratification of Douglas fir seeds. *Plant Disease*, 83:845–852

Kumria, R., Leelavathi, S., Bhatnagar, R.K. and Reddy, V.S. 2003. Regeneration and genetic transformation of cotton: present status and future perspectives. *Plant Tissue Culture*, 13:211–225

Lizarraga-Paulin, E. G., Miranda-Castro, S. P., Moreno-Martinez, E., Lara-Sagahon, A. V. and Torres-Pacheco, I. 2013. Maize seed coatings and seedling sprayings with chitosan and hydrogen peroxide: their influence on some phenological and biochemical behaviors. *Journal of Zhejiang University of Science B (Biomed Biotechnol),* 14:87–96

Miche, M. and Balandreau, J. 2001. Effects of rice seed surface sterilization with hypochlorite on inoculated Burkholderia vietnamiensis. *Applied Environmental Microbiology*, 67:3046–3052

Molan, Y.Y., Ibrahim, Y. E. and Al-Masrahi, A. A. 2010. Identification in Saudi Arabia of *Pseudomonas corrugata*, the tomato pith necrosis pathogen, and assessment of cultivar resistance and seed treatment. *Journal of Plant Pathology*, 92 (1), 213-218

Popoola, A. R., Durosomo, A. H., Afolabi, C. G. and Idehen, E. O. 2015. Regeneration of Somaclonal Variants of Tomato (*Solanum lycopersicum* L.) for Resistance to Fusarium Wilt, *Journal of Crop Improvement*, 29:5, 636-649, DOI:10.1080/15427528.2015.1066287

Richardson, M. J. 1990. *An Annotated List of Seed Borne Diseases*, 4th edition. Publisher: Zurich, Switzerland: International Seed Testing Association (ISTA), 1 v.

Sen, M. K., Jamal, M. A. H. M. and Nasrin, S. 2013. Sterilization factors affect seed germination and proliferation of *Achyranthes aspera* cultured *in vitro*. *Environmental and Experimental Biology*, 11: 119–123

Statistical Analysis System (SAS) Institute Inc. 2003. System Requirements for SAS 9.1.3 Foundation for Microsoft Windows, Cary, NC: SAS Institute Inc.

Wang, Z. F., Wang, J. F., Bao, Y. M., Wu, Y.Y., and Zhang, H. S. 2011. Quantitative trait loci controlling rice seed germination under salt stress. *Euphytica*, 178: 297-307.