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Efficacy of plant extracts, traditional materials and antibacterial chemicals against *Xanthomonas campestris* pv. *vesicatoria* on tomato seed

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The potency of mustard and ginger rhizome extracts, lemon juice, Atella (residue of traditional Ethiopian beer) and cow urine in controlling tomato seed borne pathogen, *Xanthomonas campestris* pv. *vesicatoria* (Xcv) was evaluated. Artificially inoculated seeds were treated by dipping for 30 min, 3 h, 12 h or 24 h. Streptomycin (0.5%), sodium hypochlorides (1%) and control treatments were similarly applied. The test organism was isolated from tomato fruit collected from central Rift Valley of Ethiopia; it belonged to T1P2 race group of Xcv. Tomato seeds were inoculated with Xcv suspensions, dried and dipped at room temperature in the treatments. Atella at all treatment durations completely inactivated (0 cfu/ml) Xcv from inoculated tomato seeds and induced 92 to 98% seed germination. Ginger and mustard extracts showed 0 cfu/ml on Xcv with 24 h soaking, 97 to 81% germination and 2303 to 2270 vigor indexes of tomato seed. A promising bactericidal effect of Atella is an excellent clue of using other traditional alcoholic drink residues in controlling Xcv on tomato seeds for small scale farming.

Key words: seed treatment, *Xanthomonas campestris* pv. *vesicatoria,* plant extracts, biocontrol.

INTRODUCTION

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Xcv), is a serious seed borne disease of tomato. It is destructive to tomato seedlings resulting in total crop losses in warm and humid areas due to persistence of the causal bacterium and weather conditions favorable to the disease (Sun et al., 2002). It reduces seed germination and causes defoliation of leaves. Losses of fruit yield are greatest when infection occurs early and it has been reported to account for up to 52% weight loss in infected fruits (Jones et al., 1986). The pathogen can infect both tomato and pepper (O'Garro, 1999) causing bacterial spot disease. Symptoms of bacterial spot appear on leaves, flowers, petioles, stems and roots. Infection on leaves causes defoliation, resulting in reduced marketable fruit weight and increased exposure of fruits to sun scald. Even though fruit lesions are only superficial, they reduce quality for sale fresh and also for processing. Although the pathogen can have effect on the leave, stem and fruit of tomato, its effect on germination of seed is nil (Bashan, 1986). Fruit symptoms are more distinctive than leaf or stem symptoms. Spots on green fruit first appear as black, raised, pimple-like dots surrounded by water-soaked areas. As the spots enlarge to 0.64 to 1.27 cm, they become gray-brown and scabby with sunken, pitted centers (Gleason and Edmunds, 2006). The bacterium, Xcv, is seed borne (Kucharek, 2000). It can survive on tomato seeds for periods of at least 10 years (Bashan et al., 1982). The bacteria contaminate the seeds during extraction.

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Abbreviation: Xcv, *Xanthomonas campestris* pv. *Vesicatoria.*

Currently, various antimicrobial chemicals are used to treat seeds. Antibiotics and copper compounds were used but the effective disease control options are limited with copper-based bactericides (Balestra et al., 2009). However, the use of synthetic chemicals is increasingly restricted because of public concern over toxic residues which could have adverse health and environmental effects. Moreover, they are not affordable or accessible to vegetable growers in developing countries (Rahman et al., 2008). Hot water seed treatments are also used against many bacterial and fungal seed borne diseases. Precise control of temperature and duration is critical, as seeds may be damaged by the treatment and the pathogen may not be completely eliminated (Nega et al., 2003).

Traditional antimicrobial materials offer a potential to develop alternative methods that are economical and suited for adoption by the small-scale tomato growers. Different medicinal plant extracts are used by African farmers to treat human and livestock diseases. They are cheap and can be produced locally. Such medicinal plants are suitable candidates in searching for effective antimicrobials. It has been documented that cow urine, ginger, lemon and mustard have antibacterial activity (Sathasivam et al., 2010; Stoilova et al., 2007; Rahman et al., 2011).

This study reports on the effect of extracts of Ethiopian mustard (*Brassica carinata* A. Braun, variety Yellow Dodola) and ginger rhizome (*Zingiber officinale*), lemon juice (*Citrus limon* L.), Atella (residue of traditional Ethiopian beer "Tella") and cow urine on Xcv viability and germination of tomato seed while comparing the efficacy with known antimicrobial chemicals, namely, streptomycin and sodium hypochloride.

MATERIALS AND METHODS

Inoculum preparation and inoculation of seed

An isolate of Xcv which belongs to TIP2 race (J. B. Jones, personal communication) was used. The isolate was originally obtained from symptomatic tomato fruit from central Rift Valley of Ethiopia and it was the isolate which showed more severe infection on tomato plants. The inoculum was prepared by incubating the isolate for 24 h on sucrose peptone agar and suspending the bacteria in sterile distilled water at a concentration of approximately 10^8 cells ml^{-1} adjusted using a spectrophotometer (0.15 absorbance at 600 nm) (Abbasi and Lazarovits, 2006).

Tomato seeds of variety 'Marglobe' which is susceptible to Xcv were inoculated by placing 50 g of seed in 200 ml Xcv inoculum under vacuum using vacuum pump (FJC 6909 3.0 CFM Auto AC Vacuum Pump). The seeds were soaked for 30 min and the vacuum was broken abruptly to favor penetration of the bacteria into the seed cavities. Seeds were placed in sterile Petri dishes and allowed to dry for 3 h in a laminar flow hood (Kritzman, 1991).

Test extract preparation

Ethiopian mustard was obtained from Kulumssa Agricultural Research Center. Ginger rhizome was bought from local market.

Fifty gram of mustard seed and ginger rhizome were washed with sterile distilled water and blotted with paper towels. Samples were blended in 250 ml water for 2 min. The blended plant material was filtered with cheese cloth and the extracts were centrifuged at 6000 x *g* for 10 min (Balestra et al., 2009). Fresh lemon was bought from the market and pressed. The juice of lemon was centrifuged at 6000 x *g* for 10 min. Atella was collected from nearby village that has prepared the traditional drink, Tella, and filtered through cheese cloth. Cow urine was collected from the dairy farm of Haramaya University. The filtrates and cow urine were separately sterilized using ultra filtration (0.45 μm Millipore type HAWP) and used in the experiment.

Treatments and experimental procedures

A total of 32 treatments were evaluated on tomato seed that had been artificially inoculated with Xcv isolate. Atella, cow urine, ginger rhizome extract, mustard extract, lemon juice, 0.5% streptomycin, 1% sodium hypochloride, and the control (sterile distilled water) were applied separately by dipping the seed for 30 min, 3 h, 12 h, and 24 h. Split plot design with three replication was used. The main plots were represented by the four durations of temperature and the subplots consisted of the eight antimicrobial treatments including the control. The treated seeds were allowed to dry in a laminar flow hood for 3 h.

Seed germination and seedling vigor tests

Fifty (50) treated seeds from each experimental unit were incubated in Petri dish containing sterilized moistened blotter paper. Tomato seeds were placed in the dark at 20°C to germinate (Jones, 1999). Germination percentage was recorded after 10 days. For determination of seedling vigor, seedling length was measured and vigor index was calculated using the following formula according to Abdul-Baki and Anderson (1973):

Vigor index = mean of seedling length (cm) x percentage of seed germination.

Bacterial isolation and count

The procedure by Mcguire et al. (1986) was followed for isolation of the pathogen from the seeds. One gram of treated tomato seeds was placed in 250 ml Erlenmeyer flask containing 100 ml peptone buffer. The peptone buffer was prepared by combining 5.30 g of KH_2PO_4, 8.61 g of Na_2HPO_4 and 1 g of Bacto-peptone in a litre of water. The seeds were incubated in the buffer without shaking at 4°C for 3 h. Thereafter, the seeds were agitated on a shaker for 1 h at room temperature to dislodge the bacteria, then the bacterial suspension was serially diluted in saline buffer. From each dilution, 100 µl suspensions were spread over the semi-selective culture medium, SX agar. SX agar was prepared from 10 g of soluble potato starch, 1 g of beef extract, 5 g of NH_3Cl, 2 g of KH_2PO_4, 15 g of agar, 1 ml of methyl violet, 2 ml of methyl green and 5 ml of cycloheximide. After three to five days of incubation at 28°C, colony forming units (cfu/ml) were calculated.

Data analysis

The data was set out as a split plot design using SAS software (SAS Institute, version 9.1, Cary, NC). The data was subjected to analysis of variance and the treatment means were used with least significant differences.

Table 1. Effect of treatments applied for different durations on the viability of Xcv (Log $_{10}$ cfu/ml).

Antimicrobial treatment	Time				Mean*
	30 min	3 h	12 h	24 h	
Atella	0^{b}	0^{c}	0^{b}	0^{b}	0^{E}
Cow urine	6.33^{a}	4.48^{b}	1.69^{b}	0^{b}	3.13^{C}
Ginger	2.2^{b}	1.49^{c}	0^{b}	0^{b}	0.92^{D}
Lemon juice	0^{b}	0^{c}	0^{b}	0^{b}	0^{E}
Mustard	7.38^{a}	6.64^{a}	6.44^{a}	0^{b}	5.11^{B}
Streptomycin	0^{b}	0^{c}	0^{b}	0^{b}	0^{E}
Bleach	0^{b}	0^{c}	0^{b}	0^{b}	0^{E}
Control	5.33^{a}	5.21^{ab}	6.47^{a}	8.51^{a}	6.38^{A}
CV%	53.5				

0 indicates that no bacteria were detected. Means in the two way table followed by same lower case letters in each column are not significantly different at 5% level of significance. *Means values of means followed by same uppercase letters in column are not significantly different at 5% level of significance.

RESULTS

All antibacterial treatment applications significantly affected the viability of Xcv, germination and vigor index of tomato seeds with various soaking durations. There was a significant difference between soaking duration and antimicrobial treatments independently and with combined interactions.

Antibacterial treatment effect on viability of Xcv

Atella, lemon juice, bleach and streptomycin fully inhibited Xcv with no recovery of the bacterium from tomato seed, that is, 0 cfu/ml in all soaking durations (Table 1). Soaking seed in ginger extract for 30 min and 3 h resulted in lower inhibition of Xcv (with recovery of 2.2 cfu/ml and 1.49 cfu/ml) than at 12 h and 24 h durations which completely inactivated Xcv (0 cfu/ml). Seed soaked in cow urine for 30 min, 3 h and 12 h yielded Xcv recovery of 6.33, 4.48 and 1.69 cfu/ml, respectively, but as the duration of treatment increased to 24 h, the cfu/ml was reduced to nil. Similarly, the recovery of Xcv was decreased by mustard extract from 7.38 cfu/ml at 30 min, through 6.64 cfu/ml at 3 h to 6.44 cfu/ml at 12 h without significant difference among the soaking durations. At 24 h soaking, Xcv was completely inactivated by the mustard extract. In the control treatment, Xcv population showed increase from 5.33 to 8.51 cfu/ml as the soaking duration increased from 30 min to 24 h.

Antibacterial treatment effect on seed germination and seedling vigor of tomato

Regardless of the soaking duration, treating tomato seed in Atella and ginger extract did not significantly affect seed germination over the control (93%); mean seed germination of 95.34 to 96.66% were recorded for these treatments (Table 2). On the other hand, lemon juice and streptomycin significantly ($P < 0.05$) reduced seed germination in all treatment durations. Germination of seed treated with lemon juice ranged from 9.33% for 24 h to 22.67% for the 30 min duration. Likewise 33.33% and 2.67% of seed germinated when treated with streptomycin for 30 min and 24 h, respectively. Seed soaked in bleach for 24 h had significantly lower seed germination (25.33%) in comparison with the control (92%). The control treatment (without antibacterial treatment) tomato seeds germinated well in all soaking durations.

The lowest seedling vigor index was observed for streptomycin (9 at 24 h) and lemon juice (14 at 24 h) with no significant difference among the soaking durations (Table 3). Ginger extract showed low vigor index at 24 h soaking duration, but at 30 min, 3 h and 12 h soaking durations, vigor index was high (2422 to 2303) as well as the control (2258 to 2350). Similarly, bleach at 30 min and 3 h soaking showed the highest vigor index (2215) like the control. Seeds treated with Atella presented an average vigor index value ranging from 1570 at 30 min soaking to 1665 at 12 h soaking durations. The highest vigor index (2479 to 2270) was observed with ginger and mustard extracts at 30 min, 3 h and 12 h soaking durations. When the time of soaking was prolonged from 12 to 24 h, all the antimicrobial treatments resulted in significantly lower vigor index values comparatively to the other soaking durations. In the control treatment, tomato seeds vigor index was the highest in all soaking durations.

DISCUSSION

The use of commonly found plant extracts and traditional materials to manage seed borne pathogens is promising. The overall objective of this experiment was to evaluate

Table 2. Mean germination (%) of tomato seeds soaked in different treatments for different durations.

Antimicrobial treatment	Time				Mean*
	30 min	3 h	12 h	24 h	
Atella	96^{a}	94.67^{a}	98.67^{a}	92^{a}	95.34^{AB}
Cow urine	89.33^{a}	85.33^{a}	80^{c}	74.67^{b}	82.33^{C}
Ginger	96^{a}	97.33^{a}	97.33^{ab}	96^{a}	96.66^{A}
Lemon juice	22.67^{b}	26^{b}	12^{e}	9.33^{d}	17.50^{E}
Mustard	97.33^{a}	94.67^{a}	92^{b}	81.33^{b}	91.33^{B}
Streptomycin	33.33^{b}	26^{b}	16^{e}	2.67^{d}	19.50^{E}
Bleach	89.33^{a}	88^{a}	69.33^{d}	25.33^{c}	68.00^{D}
Control	92^{a}	93.33^{a}	94.67^{ab}	92^{a}	93.00^{AB}
CV%	8.55				

Means in the two way table followed by same lower case letter(s) within a column are not significantly different at 5% level of significance. *Means values of means followed by same uppercase letters in column are not significantly different at 5% level of significance.

Table 3. Mean vigor index of tomato seeds soaked in different treatments for different durations.

Antimicrobial treatments	Time				Mean*
	30 min	3 h	12 h	24 h	
Atella	1570^{b}	1577^{b}	1665^{b}	1536^{c}	1587^{CD}
Cow urine	1635^{b}	1616^{b}	1459^{c}	1355^{d}	1516^{D}
Ginger	2422^{a}	2364^{a}	2303^{a}	371^{f}	1865^{B}
Lemon juice	61^{c}	56^{c}	25^{d}	14^{g}	39^{E}
Mustard	247^{a}	2401^{a}	2270^{a}	1995^{b}	2287^{A}
Streptomycin	128^{c}	90^{c}	51^{d}	9^{g}	69^{E}
Bleach	2215^{a}	2200^{a}	1673^{b}	573^{e}	1666^{C}
Control	2258^{a}	2296^{a}	2350^{a}	2275^{a}	2295^{A}
CV%	9.55				

Means in the two way table followed by same lower case letter(s) in each column are not significantly different at 5% level of significance. *Means values of means followed by same uppercase letters in column are not significantly different at 5% level of significance.

the effect of extracts of Ethiopian mustard, ginger rhizome, lemon juice, Atella and cow urine on Xcv viability on tomato seed and their effect on germination of tomato seed by comparing the efficacy with streptomycin and sodium hypochloride. The result shows that all the antimicrobial treatments used inhibited the growth of Xcv on the seeds of tomato at prolonged soaking duration while for lemon juice, cow urine, ginger, streptomycin and bleach prolonged soaking durations significantly inhibited the seed germination and vigor index. On the other hand, Atella and mustard showed less effect on seed germination and vigor index of tomato seeds at all soaking durations that guarantee the negative effect of soaking but mustard inhibitory effect on Xcv viability was low. Hence among the antimicrobial treatments used, Atella was found to be the best. Atella is shown to be more effective in controlling Xcv with all soaking durations and having least effect on germination of tomato seeds. Atella is the residue of fermentation process in preparation of Tella, a traditional alcoholic drink. Samples of Tella analyzed earlier contained 6.07% ethyl alcohol (Desta-Belachew, 1977). Since Atella is a complex material with little information on specific chemical constituents, its alcohol content is expected to contribute to first-rate inhibition effect on Xcv. Alcohol denatures the lipid in the cell membrane of bacteria; specifically it has significant effect on Gram negative bacteria. This fact could explain why Atella has high effect on disinfection of Xcv on the tomato seeds and least effect on the germination of the tomato seeds. It needs further study to identify the non-alcoholic inhibitory effect of Atella on Xcv.

Lemon juice completely inhibited Xcv in all soaking durations. Similarly, the antibacterial effect of lemon juice was reported earlier (Rahman et al., 2011; Onyeagba et al., 2004; Oboh et al., 1992). The antimicrobial potency of

plants is due to tannins, saponins, phenolic compounds, essential oils and flavonoids. Lemon juice is efficient against multi-drug resistant *Escherichia coli* (Rahman et al., 2011). Lemon juice and leaves extract inhibit the growth of *Pseudomonas aeruginosa* and *E. coli*, respectively. Moreover, *Staphylococcus aureus, Bacillus* spp. and *Salmonella* spp. are susceptible to lemon juice (Onyeagba et al., 2004). On the other hand, pH values lower than 3 and higher than 8 are reported to inhibit seed germination (Jansen and Cronin, 1953) and lemon juice has approximate pH values of 2 to 2.5; the acidic nature of lemon juice might be the reason for the low seed germination and seedling vigor index values. Reducing the concentration of lemon juice could change their effect on germination of tomato seed.

Ginger contains gingerols and polyphenol compounds (antioxidants), which have many medicinal properties and is effective against many diseases that affect cultivated crops (Stoilova et al., 2007; Park et al., 2008). Prolonged soaking in ginger extract results in the inhibition of Xcv on the tomato seeds. Azu and Onyeagba (2007) found that extract of ginger inhibited *E. coli* and *Salmonella typhi.* Ginger extract is also shown to be as effective as synthetic pesticides such as benomyl in reducing bacterial leaf spot disease severity on *Solanum gilo* and *Solanum torvum* (Opara and Obani, 2009). Ginger extract has excellent effect on germination of tomato seeds with high vigor index like the control. The effect of ginger extract on the germination of seeds studied by Hasan et al. (2005) showed that soaked wheat seeds in ginger extract resulted in 10% increased in seed germination and highest vigor index over the control. The ability of the extract to improve seed germination could be attributed to the suppression of seed borne diseases that could have damaged the embryo of the seeds.

The slightest inhibition effect is observed with cow urine and mustard extract. Both chemicals inhibited Xcv at 24 h soaking time. This shows that the antibacterial chemicals in cow urine and mustard were found in small amount of concentration. Sathasivam et al. (2010) studies shows that cow urine distillate has antibacterial and antifungal activities that can be used in the control of bacteria and fungi of various origins. Hence to improve the efficiency of cow urine on Xcv, cow urine distillate could be used. According to Kumari (2006), phenols are present in cow urine and they are bactericidal to gram positive and gram-negative bacteria. Therefore, presence of phenols in cow urine may be instrumental for its potent antimicrobial nature. Mustard contains allyl isothocyanate that are sulfur-containing compounds. Brassica crops (such as mustard) are reported to have fungicidal activities (Brown and Morra, 2005) but the bactericidal activity is reported to be limited. Rahman et al. (2010) described that mustard extract has showed inhibitory activity against *S. aureus* but not on *Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus faecalis*, *Micrococcus luteus*, *E. coli* and *Candida albicans.*

Bleach and streptomycin have complete inhibition effect on the Xcv inoculated tomato seeds. Bleach and streptomycin are known worldwide and accepted that they kill most bacterial disease-causing organisms on or within seeds. A study by Mensah et al. (2012) shows that as the concentration of streptomycin increases, the percentage of germination decreases which correlates with the present finding which shows a negative effect on the germination of tomato seeds.

The control treatment showed that the presence of Xcv on the tomato seeds had no effect on the vigor index and germination of tomato seeds. This result coincides with Bashan (1986) experiment which showed that Xcv had no effect on germination of tomato seeds while it disagree with Kavitha and Umesha (2007). Bashan (1986) found out that the effect of Xcv on the germination of tomato varies with Xcv strains from different source and countries which could be the reason for the Ethiopian strain nil effect on tomato seed germination.

Conclusion

Atella has a potential antibacterial effect on tomato seed borne pathogen, Xcv, without affecting seed germination. An excellent bactericidal effect of Atella against Xcv shows the promising result of other fermented alcoholic residues to manage bacterial seed borne pathogens. Moreover, ginger extract and mustard extract can be used as an alternative to chemicals for seed treatment against Xcv with restricted soaking duration. It is recommended that to sustain the *in vitro* study, further *in vivo* study must be undertaken. Use of antimicrobials which are commonly found around the habitation of rural areas can be used to reduce the cost of chemicals and over reliance of small scale farmers on agricultural chemicals.

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