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DISEASE CONTROL

The effect of plant extracts as seed treatments to control bacterial leaf spot of tomato in Tanzania

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Abstract Bacterial leaf spot (BLS) caused by seed-borne xanthomonads is a serious disease of tomato (*Solanum lycopersicum* L.), causing significant losses in both yield and quality. To identify more effective control measures, we evaluated crude extracts from 84 plant species in in vitro and in planta assays for antibacterial activity against BLS of tomato. In the in vitro assays, 20.2 % of the tested plant extracts totally inhibited growth of bacteria when seed washings from treated seeds were plated on nutrient agar medium. In the in planta assays, 17.8 % of the tested plant extracts reduced BLS incidence by 100 % in tomato seedlings. The most effective seed treatments were obtained with extracts from *Aloe vera*, *Betula pendula*, *Coffea arabica*, *Glycyrrhiza uralensis*, *Juniperus communis*, *Ocimum basilicum*, *Quercus robur*, *Rheum palmatum*, *Rosmarinus officinalis*, *Ruta graveolens*, *Sinapis alba*, *Yucca schidigera* and *Salvia officinalis*. Seed treatment of tomato with these extracts completely inhibited *Xanthomonas perforans* in both in vitro and in planta assays. Extracts from *A. vera*, *C. arabica* and *Y. schidigera* were tested three times using tomato seeds of cultivars Tanya, Cal-J and Moneymaker in Tanzania. Treatment of tomato seeds with these extracts had a positive effect on the number of normal seeds and had no effect on seedling vigor, height and weight. These results indicate that plant extracts from *A. vera*, *C. arabica* and *Y. schidigera* are potential candidates for seed treatment against seed-borne xanthomonads of tomato in Tanzania.

Keywords Plant extracts · Antibacterial activity · *Xanthomonas perforans* · Tomato · Seed treatment

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Introduction

Bacterial leaf spot (BLS) incited by *Xanthomonas euvesicatoria*, *X. vesicatoria*, *X. perforans* and *X. gardneri* (Anonymous 2006; Jones et al. 2004) is a serious disease of tomato (*Solanum lycopersicum* L.) that occurs worldwide in regions with warm and humid climates (Jones et al. 2000; Stall et al. 1994; Tamir-Ariel et al. 2007). The BLS xanthomonads also affect pepper (*Capsicum* spp.), reducing both fruit yield and quality. Recently, *X. campestris* pv. *raphani* (Punina et al. 2009) and *X. arboricola* (Myung et al. 2010; E. R. Mbega et al., unpublished data) were also reported as pathogens of tomato. The BLS xanthomonads can survive in seeds, plant debris and volunteer plants (Kaaya et al. 2003). Seed infection by BLS pathogens of about 40.7 % has been reported in Tanzania, causing yield losses up to 45 % (Black et al. 2001; Kaaya et al. 2003).

Management of BLS is limited to foliar applications of copper-based compounds. However, presence of strains of BLS pathogens with a high degree of tolerance to copper (Carrillo-Fasio et al. 2001; Gitaitis et al. 1992; Gore and O'Garro 1999; Lee and Cho 1996; Martin et al. 2004; Scheck et al. 1996; Shenge et al. 2007) and the considerable number of *Xanthomonas* species and races causing BLS symptoms in tomato and pepper (Jones et al. 2004) have made the control of the disease difficult.

Plants synthesize a number of compounds with antibiotic and antimicrobial properties (Amadioha 2003; Kumar and Parmar 1996; Opara and Wokocha 2008; Prakash and Rao 1997). Such compounds can therefore, be exploited as an alternative approach to manage or control BLS. Some of the known advantages of using pesticides of plant origin to control plant diseases include low mammalian toxicity, minimum health hazards and environmental pollution, and low risks of development of resistance by pathogens (Amadioha 2003; Kumar and Parmar 1996; Prakash and Rao 1997). Biopesticides may also be less expensive, easily available (because of their natural occurrence) and depending on the concentration, they may have no effects on seed viability, plant growth and food quality (Opara and Wokocha 2008).

In the present study, we applied crude extracts from a large number of plant species to tomato seeds to evaluate their potential to control BLS. The effect of the most promising extracts on seed germination and tomato seedling growth was also investigated.

Materials and methods

Seed samples

Tomato (*S. lycopersicum* L.) seeds of cultivars Cal-J, Tanya and Moneymaker, collected from tomato growers in Tanzania, were tested for infection as described by International Seed Federation (2007). Seed samples that were free of infection by *Xanthomonas* spp. were used in the experiments. One thousand seeds per cultivar were surface-disinfested in 70 % ethanol for 1 min, then in 1 % sodium hypochlorite for 3 min and rinsed three times in sterile distilled water. The seeds were then transferred to Petri dishes containing sterile filter papers and allowed to air-dry overnight in a laminar flow chamber and stored at 4 °C until used.

Seed inoculation with bacterial suspensions

Surface-disinfested seeds of tomato cultivar Tanya were inoculated with *X. perforans* strain NCPPB 4321 based on its ability to cause severe BLS symptoms on various tomato cultivars, including those grown in Tanzania. Inoculum was prepared from 24-h-old bacterial cultures grown on nutrient agar (NA) medium (meat extract 3 g, Bacto peptone 5 g, Bacto agar 20 g, distilled water 1000 mL) at 28 °C. Bacterial cultures were flooded with 10 mL of sterile distilled water and gently scraped with a flamed Drigalski spatula. The inoculum suspension was then homogenized using a vortex mixer and suspended in sterile distilled water to obtain a ca. 10^8 CFU/mL ($OD_{600} = 0.01$) (NanoDrop, Thermo Fisher Scientific, Beverly, MA, USA). One thousand seeds of tomato were vacuum-infiltrated for 30 min with 10 mL of the bacterial suspension, and seeds were air-dried in the laminar air flow chamber at 4 °C until used.

Seed treatment with plant extracts

Plant for extractions were obtained from companies in Denmark and farmers in Tanzania as shown in Table 1. Two grams of plant material were suspended in 20 mL sterile distilled water in a 50 mL conical flask to obtain a 10 % (w/v) concentration. The conical flasks with the suspensions were briefly heated on a hot electric plate until boiling and cooled for 5 min. The suspensions were filtered through sterile cheesecloth, and the extracts were autoclaved at 115 °C for 15 min and kept at 4 °C until used. Twenty tomato seeds pre-inoculated with *X. perforans* were treated with 1 mL of the 10 % plant extract in an Eppendorf tube and placed on an agitation table at 100 rpm overnight at 25 °C. Untreated, seeds treated with copper sulphate (200 μg/mL $CuSO_4{\cdot}5H_2O$) or sterile distilled were included as controls. After overnight incubation, the treated seeds were blot-dried and allowed to air-dry for 1 h in the laminar air flow cabinet.

Evaluation of antibacterial activity of plant extracts in vitro

Washing samples (100 μL) from treated seeds were collected using sterile pipettes and serially diluted to 10^{-2} with sterile distilled water in Eppendorf tubes. An aliquot of 100 μL from each dilution was spread onto 0.6 cm thick NA medium in Petri dishes (diameter 8.5 cm × depth 1.3 cm) using a sterile glass rod. The plates were incubated at 28 °C. A pure culture of *X. perforans* NCPPB 4321 was included as a control. Yellow colonies with morphology and color similar to those of *X. perforans* were counted after 96 h. The identity of suspected colonies from each plate was confirmed by pathogenicity tests. The abaxial surfaces of four 14-day-old plants of tomato cultivar Tanya were sprayed to runoff with an inoculum suspension of 10^8 CFU/mL ($OD_{600} = 0.01$) prepared from 24-h-old bacterial cultures grown on NA at 28 °C. The inoculated seedlings were covered with polyethylene bags and kept in the growth chamber for 14 days. Seedlings sprayed with sterile saline water (containing 0.85 % of NaCl) were used as a negative control, and seedlings sprayed with suspensions prepared from the *X. perforans* culture served as positive controls. The plants were examined for symptoms 14 days after inoculation and scored as negative when no obvious symptoms were observed. Leaves with water-soaked lesions that developed into dark brown spots were scored as positive for BLS disease.

Table 1 Origin of plant extracts and control of bacterial leaf spot (BLS) of tomato caused by *Xanthomonas perforans* after seed treatment with extracts or control treatments

Common name	Scientific name	Family	Part tested	Origin	CFU/mL	BLS-RI (%)[a]
Aloe	*Aloe vera* L.	Aloaceae	Stem	NN, DK	0.0×10^0	100
Silver birch	*Betula pendula* Roth.	Betulaceae	Leaf	NDC, DK	0.0×10^0	100
Coffee	*Coffea arabica* L.	Rubiaceae	Seed[b]	Tanzania	0.0×10^0	100
Licorice	*Glycyrrhiza uralensis* L.	Fabaceae	Stem/leaf	NDC, DK	0.0×10^0	100
Juniper	*Juniperus communis* L.	Cupressaceae	Stem	NDC, DK	0.0×10^0	100
Basil	*Ocimum basilicum* L.	Lamiaceae	Leaf	NDC, DK	0.0×10^0	100
Oak	*Quercus robur* L.	Fagaceae	Bark	NN, DK	0.0×10^0	100
Rhubarb	*Rheum palmatum* L.	Polygonaceae	Stem	NN, DK	0.0×10^0	100
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Stem	NN, DK	0.0×10^0	100
Rue	*Ruta graveolens* L.	Rutaceae	Stem	NDC, DK	0.0×10^0	100
White mustard	*Sinapis alba* L.	Brassicaceae	Root	NDC, DK	0.0×10^0	100
Mojave yucca	*Yucca schidigera* L.	Agavaceae	Stem	NDC, DK	0.0×10^0	100
Sage	*Salvia officinalis* L.	Lamiaceae	Stem	NDC, DK	0.0×10^0	100
Nor-grape 80	*Vitis vinifera* L.	Vitaceae	Stem/leaf	NDC, DK	0.0×10^0	94
Punica	*Punica granatum* L.	Punicaceae	Stem	NDC, DK	0.0×10^0	86.7
Olive	*Olea europaea* L.	Oleaceae	Leaf	NDC, DK	0.0×10^0	81.2
Clove	*Caryophyllus aromaticus* L.	Myrtaceae	Twig	NDC, DK	0.0×10^0	75
White willow	*Salix alba* L.	Salicaceae	Bark	NN, DK	2.0×10^0	100
Grape vine	*Vitis vinifera* L.	Vitaceae	Leaf	NN, DK	1.0×10^0	79.8
Quinoa	*Chenopodium quinoa* Wild.	Chenopodiaceae	Stem	NN, DK	1.8×10^3	75
Soapwort	*Saponaria officinalis* L.	Caryophyllaceae	Stem/leaf	NN, DK	2.2×10^1	100
Ginkgo	*Ginkgo biloba* L.	Ginkgoaceae	Leaf	NDC, DK	3.3×10^4	94
Daisy	*Bellis perennis* L.	Asteraceae	Flower	NDC, DK	4.0×10^4	90.1
Inula	*Inula helenium* L.	Asteraceae	Stem	NDC, DK	1.0×10^1	86.7
Rosemary	*Rosmarinus officinalis* L.	Lamiaceae	Leaf	NN, DK	2.5×10^3	81.6
Tea	*Camellia sinensis* L.	Theaceae	Leaf	NDC, DK	1.0×10^1	81.2
Bilberry	*Vaccinium myrtillus* L.	Vacciniaceae	Leaf	NN, DK	1.0×10^1	81.2
Celery	*Apium graveolens* L.	Apiaceae	Root	NDC, DK	1.0×10^1	80
Ginseng	*Panax ginseng* C.A. Meyer.	Araliaceae	Stem/leaf	NDC, DK	1.0×10^1	80
Burnet-saxifrage	*Pimpinella saxifraga* ssp. *nigra* (Mill)	Apiaceae	Stem	NN, DK	1.0×10^6	80
Thyme	*Thymus vulgaris* L.	Lamiaceae	Leaf/stem	NDC, DK	3.9×10^3	80
Maypop	*Passiflora incarnata* L.	Passifloraceae	Flower	NN, DK	6.0×10^4	79.8
Red pepper	*Capsicum frutescens* L.	Solanaceae	Fruit	NN, DK	1.8×10^5	73.4
Chamomile	*Matricaria chamomilla* L.	Asteraceae	Flower	NN, DK	2.0×10^1	73.3
Neem	*Azadirachta indica* L.	Meliaceae	Seed	Tanzania	7.0×10^2	68.7
Quinine	*Cinchona pubescens* Vahl.	Rubiaceae	Bark	NDC, DK	9.0×10^1	66.7
High mallow	*Malva sylvestris* L.	Malvaceae	Leaf	NN, DK	9.8×10^3	66.6
Cowslip	*Primula veris* L.	Primulaceae	Stem	NN, DK	6.0×10^1	60
Sisal	*Agave sisalana* Perrine.	Agavaceae	Root	Tanzania	$>1.0 \times 10^7$	59.9
Agapanthus	*Agapanthus* sp.	Alliaceae	Leaf	NDC, DK	1.9×10^5	53.2
Sisal	*Agave sisalana* Perrine.	Agavaceae	Leaf	Tanzania	2.8×10^5	53.2
Couch grass	*Agropyron repens* (L.) Beauv.	Poaceae	Stem	NDC, DK	1.1×10^3	53.1
Elm	*Ulmus campestris* L.	Ulmaceae	Bark	NN, DK	5.5×10^2	49.5
Sisal	*Agave sisalana* Perrine.	Agavaceae	Stem	Tanzania	1.9×10^5	46.8
Horsetail	*Equisetum arvense* L.	Equisateaceae	Leaf	ND, DK	1.6×10^5	40
Black poplar	*Populus nigra* L.	Salicaceae	Twig	NN, DK	6.0×10^0	40
Elderberry	*Sambucus nigra* L.	Caprifoliaceae	Leaf	ND, DK	6.0×10^0	40
Turmeric	*Curcuma longa* L.	Zingiberaceae	Stem	ND, DK	$>1.0 \times 10^7$	40

Table 1 continued

Common name	Scientific name	Family	Part tested	Origin	CFU/mL	BLS-RI (%)[a]
Adam's needle	*Yucca filamentosa* L.	Agavaceae	Stem	NN, DK	2.4×10^4	37.5
Onion	*Allium cepa* L.	Liliaceae	Bud	ND, DK	2.0×10^6	33.5
Eucalyptus	*Eucalyptus globulus* L.	Myrtaceae	Leaf	Tanzania	3.7×10^3	33.5
Avocado	*Persea americana* L.	Lauraceae	Fruit peels	Tanzania	$>1.0 \times 10^7$	33.5
Buckthorn	*Frangula alnus* Mill.	Rhamnaceae	Bark	ND, DK	2.0×10^1	29.9
Plantain	*Plantago major* L.	Plantaginaceae	Leaf	ND, DK	6.0×10^5	29.9
Southernwood	*Artemisia abrotanum* L.	Asteraceae	Leaves	ND, DK	5.8×10^6	26.8
Lemongrass	*Cymbopogon citratus* L.	Poaceae	Leaves	ND, DK	9.8×10^5	26.8
Eucalyptus	*Eucalyptus globulus* L.	Myrtaceae	Seeds	Tanzania	$>1.0 \times 10^7$	26.8
White clover	*Trifolium repens* L.	Fabaceae	Flower	NN, DK	2.6×10^5	26.8
Couch grass	*Agropyron repens* L.	Poaceae	Leaf	ND, DK	1.9×10^5	26.7
Silver birch	*Betula pendula* Roth.	Betulaceae	Bark	ND, DK	5.0×10^3	26.7
Scots pine	*Pinus sylvestris* L.	Pinaceae	Leaf	ND, DK	6.0×10^3	26.7
Red clover	*Trifolium pratense* L.	Fabaceae	Flower	NN, DK	6.8×10^3	26.7
Ginger	*Zingiber officinale* Rosc.	Zingiberaceae	Bud	NN, DK	7.0×10^2	26.7
Yucca	*Yucca* sp.	Agavaceae	Stem/leaf	NN, DK	2.6×10^3	25
Adam's needle	*Yucca filamentosa* L.	Agavaceae	Leaf	NN, DK	2.4×10^5	25
Oak	*Quercus robur* L.	Fagaceae	Leaf	NN, DK	7.0×10^1	18.7
Soapbark	*Quillaja saponaria* Molina.	Quillajaceae	Bark	NN, DK	2.6×10^3	18.7
Chicory	*Cichorium intybus* L.	Asteraceae	Stem	ND, DK	1.0×10^5	13.5
Water mint	*Mentha aquatica* L.	Lamiaceae	Leaf	ND, DK	4.0×10^5	13.5
Irish moss	*Chondrus crispus* L.	Gigartinaceae	Leaf	ND, DK	3.5×10^5	13.3
Tansy	*Tanacetum vulgare* L.	Asteraceae	Stem/leaf	ND, DK	1.3×10^4	6.2
Neem	*Azadirachta indica* L.	Meliaceae	Leaf	Tanzania	5.0×10^5	0
Bladderwrack	*Fucus vesiculosus* L.	Fucaceae	Flower	NN, DK	6.9×10^4	0
Coral plant	*Jatropha* sp.	Euphorbiaceae	Seed	Tanzania	2.2×10^5	0
Coral plant	*Jatropha* sp.	Euphorbiaceae	Leaf	Tanzania	1.2×10^5	0
Lantana	*Lantana camara* L.	Verbenaceae	Leaf	Tanzania	5.0×10^4	0
Spiny restharrow	*Ononis spinosa* L.	Fabaceae	Leaf	ND, DK	6.0×10^5	0
Silverweed	*Pontentilla anserine* L.	Rosaceae	Leaf	ND, DK	9.6×10^3	0
Quassia	*Quassia* sp.	Simaroubaceae	Bark	NN, DK	1.3×10^5	0
Nettle	*Urtica* sp.	Urticaceae	Leaf	ND, DK	1.4×10^5	0
Mistletoe	*Viscum album* L.	Loranthaceae	Leaf/stem	ND, DK	4.6×10^5	0
Cardamon	*Ellettaria cardamomum* L.	Zingiberaceae		ND, DK	$>1.0 \times 10^7$	−10
Alfalfa	*Medicago sativa* ssp. *sativa* L.	Fabaceae	Seed	ND, DK	$>1.0 \times 10^7$	−10
Fenugreek	*Trigonella foenum-graecum* L.	Fabaceae	Seed	ND, DK	1.3×10^6	−20
Controls						
Sterile distilled water	–	–	–	–	$>1.0 \times 10^7$	0
Copper sulphate	–	–	–	–	0.0×10^0	100
Untreated seed	–	–	–	–	0	0

– not applicable, *NN, DK* Nor-Natur, Denmark, *ND, DK* Natur Drogeriet, Denmark

[a] Bacterial leaf spot reduction index (BLS-RI) was calculated as $(C - T)/C \times 100$ %, where C is the incidence of BLS in tomato seedlings treated with sterile distilled water (negative control) and T is the incidence of BLS of tomato seedlings treated with plant extract

[b] Processed coffee (Africafe®) from Afri Tea and Coffee Blenders (1993) Ltd., Dar es Salaam, Tanzania

Evaluation of antibacterial activity of plant extracts in planta

To evaluate the effect of the plant extracts on the control of *X. perforans* in seedling assays, 16 tomato seeds treated with plant extracts as previously described were sown in pots (8 cm diameter) containing a 1:3 ratio of sterile sand and peat soil (Pindstrup substrate No. 2, Pindstrup Mosebrug A/S, Ryomgaard, Denmark) and kept in growth chamber at 28 °C under high relative humidity (>85 %). Twenty-one days after sowing, BLS incidence was assessed by calculating the percentage of seedlings with leaf spot symptoms in the total number of emerged seedlings. The efficacy of plant extract treatments in the control of BLS in tomato seedlings was calculated as the BLS reduction index (BLS-RI) = $(C - T)/C \times 100$ %, where C is the incidence of BLS in tomato seedlings raised from seeds treated with sterile distilled water (negative control) and T is the incidence of BLS in tomato seedlings from infected seeds treated with a given plant extract. In addition to the BLS incidence and reduction index, the height, mass and width of seedlings were also evaluated for the best-performing plant extracts. The height of the seedlings was determined by measuring the aerial part of the seedlings from the soil surface to the node of the terminal developing leaf. Fresh mass of the aerial plant part was determined using tomato seedlings cut at the base of the stem by a pair of scissors, and the seedling mass was weighed. To determine the width of the seedlings, we placed a digital caliper at a right angle to the seedlings and recorded the reading at the widest point of the stem. The measurements were repeated in three independent experiments. Plant extracts with the best ability to reduce BLS in tomato during the initial screening tests were determined by comparing the data obtained with Student–Newman–Kuels (SNK) test using SAS version 9.1 software (SAS Institute, Cary, NC, USA). The choice of plant extracts used for screenhouse evaluations in Tanzania was based on four criteria: (1) effectiveness in reducing BLS, (2) most normal seedlings in germination tests, (3) highest vigor index in seedling assays and (4) no phytotoxicity.

Effect of plant extracts on seed germination and seedling growth

The effect of selected plant extracts on seed germination, seedling vigor and mass was evaluated in *Xanthomonas*-free tomato seeds treated with plant extracts as previously described. Seed germination tests were conducted using 400 tomato seeds per treatment. The standard International Seed Testing Association top of paper method (ISTA 2005) was used. The seeds were plated uniformly (50 seeds per replicate) onto three layers of moist blotter paper in a plastic container kept at 27 ± 2 °C and RH >85 % for 14 days. Normal and abnormal seedlings and dead seeds were counted for the germination tests. The same seedlings were used to determine vigor and dry mass. The vigor test involved measurements of root and shoot lengths of seedlings and the percentage seed (normal seedlings) germination. The seedling vigor index (Vi) was calculated as Vi = (mean root length + mean shoot length) × (percentage germination) (Abdul-Baki and Anderson 1973). To determine the dry mass, we wrapped seedlings from each treatment in the germination tests in aluminium foil and dried them in an oven at 103 °C for 24 h. The dried seedlings were then allowed to cool to room temperature and weighed.

Evaluation of plant extracts for production of healthy tomato transplants in the screenhouse

Tomato seeds of cultivars Tanya, Cal-J and Moneymaker, collected from tomato growers in Tanzania and free of BLS-causing xanthomonads, were inoculated with *X. perforans* and treated with plant extracts as previously described. One hundred seeds per cultivar per treatment were sown in polyethylene plastic trays (56.5 × 26.5 × 6 cm) containing a mixture of forest soil, rice husks and farmyard manure (3:1:1). The trays were kept in the screenhouse at 25–33 °C and RH >85 %. Seven days after sowing, 40 seedlings per treatment of each cultivar (10 seedlings per replicate in four replications) were randomly selected and transferred to polyethylene sleeves (6.5 × 9.0 cm) containing the same growth substrate as previously described. The sleeves with the seedlings were placed on the screenhouse benches at the same temperature and RH. BLS incidence and severity in the tomato seedlings were assessed 21 days after sowing. Disease severity was determined based on the Horsfall and Barrett (1945) scale with minor modifications (Shenge 2006), where 1 = no disease, 2 = >0–3 % of leaves with BLS symptoms, 3 = >3–12 % of leaves with BLS symptoms, 4 = >12–25 % of leaves with BLS symptoms, 5 = >25–50 % of leaves with BLS symptoms and 6 = >50 % of leaves with BLS symptoms. In addition to disease incidence and severity, the height and mass of seedlings were also evaluated as already described. The experiment was repeated three times from March to September 2010.

Data analysis

In the in vitro assays, the average of the total number of colony forming units of *X. perforans* on NA was calculated based on three replications. Data for BLS incidence, severity, seed germination, seedling vigor, plant dry and fresh mass and plant height were analyzed using Proc

GLM, and mean separation tests were calculated with the Student–Newman–Kuels (SNK) using SAS version 9.1 software (SAS Institute).

Results

Results for preliminary screening of the effectiveness of plant extracts on *X. perforans* indicated that 17 of 84 tested plant extracts (20.2 %) were able to totally reduce the pathogen in in vitro assays (Table 1). The extracts were from *Aloe vera*, *Betula pendula*, processed *Coffea arabica*, *Glycyrrhiza uralensis*, *Juniperus communis*, *Ocimum basilicum*, *Quercus robur*, *Rheum palmatum*, *Rosmarinus officinalis*, *Ruta graveolens*, *Sinapsis alba*, *Yucca schidigera*, *Salvia officinalis*, *Vitis vinifera*, *Punica granatum*, *Olea europea* and *Caryophillus aromaticus*. The effect of these plant extracts on *X. perforans* was similar to that obtained when seeds were treated with copper sulphate (Table 1).

In the in planta assays, 15 of 84 plant extracts (17.8 %) completely inhibited symptoms of BLS in tomato seedlings. Such plant extracts were from *A. vera*, *B. pendula*, *C. arabica*, *G. uralensis*, *J. communis*, *O. basilicum*, *Q. robur*, *R. palmatum*, *R. officinalis*, *R. graveolens*, *S. alba*, *Y. schidigera* and *S. officinallis* (Table 1). Only 13 of 84 extracts (corresponding to 15.5 %) of the assayed plant extracts controlled BLS in both in vitro and in planta assays. These extracts were from *A. vera*, *B. pendula*, *C. arabica*, *G. uralensis*, *J. communis*, *O. basilicum*, *Q. robur*, *R. palmatum*, *R. officinalis*, *R. graveolens*, *S. alba*, *Y. schidigera* and *S. officinalis* and were selected for further experiments.

The results obtained from evaluation of the best-performing plant extracts are shown in Table 2. The 13 selected plant extracts all significantly reduced ($P < 0.001$) the incidence of BLS in tomato seedlings without significantly affecting the growth of tomato seedlings. Seed treatment with plant extracts from *A. vera*, *C. arabica*, *G. uralensis* and *Y. schidigera* totally reduced ($P < 0.001$) the incidence of BLS in tomato (Table 2). The efficacy of these plant extracts to inhibit the growth of *X. perforans* was similar to the effects obtained when seeds were treated with copper sulphate (bactericide) control and untreated (disease free) seed control.

The results also showed that, treatment of tomato seeds with the best-performing plant extracts did not negatively affect the growth of tomato seedlings compared to the treatment with copper sulphate and untreated, disease-free seeds. In contrast, seed treatment with sterile distilled water (negative control) resulted in seedlings with significantly lower ($P < 0.001$) fresh mass (0.31 g) and width (1.45 mm) compared to the other seed treatments (Table 2).

The effects of tomato seed treatment with 10 % aqueous plant extracts from *A. vera*, *C. arabica*, *G. uralensis* and *Y. schidigera* on seed germination, seedling vigor and dry mass is summarised in Table 3. Treatment of tomato seeds with extracts from *A. vera*, *C. arabica* and *Y. schidigera* significantly increased ($P < 0.05$) the number of normal

Table 2 Effect of seed treatment with selected plant extracts on incidence of bacterial leaf spot caused by *Xanthomonas perforans* and on growth of tomato seedlings of cultivar Tanya

Treatment	Incidence (%)[a]	Height (cm)	Mass (g)	Width (mm)
Control				
Sterile distilled water	83.30a	13.50b	0.31d	1.45d
Copper sulphate	0.00e	16.13a	0.85abc	1.81abc
Untreated seed	0.00e	15.98a	0.75bc	1.78c
Plant extract				
Aloe vera	0.00e	17.28a	0.96abc	2.05ab
Betula pendula	18.80b	16.90a	0.83abc	2.00abc
Coffea arabica	0.00e	17.13a	0.98ab	2.04ab
Glycyrrhiza uralensis	0.00e	17.23a	0.96abc	2.06ab
Juniperus communis	6.30d	16.11a	0.72c	1.97abc
Ocimum basilicum	18.80b	16.84a	0.78abc	1.93abc
Quercus robur	18.80b	16.69a	0.82abc	1.97abc
Rheum palmatum	18.80b	16.50a	0.82abc	2.04ab
Rosmarinus officinalis	18.80b	16.06a	0.78abc	1.98abc
Ruta graveolens	18.80b	16.16a	0.49ab	1.82abc
Salvia officinalis	12.50c	16.56a	0.82abc	2.04ab
Sinapis alba	12.50c	16.13a	0.74c	1.90abc
Yucca schidigera	0.00e	17.24a	1.00a	2.13a
Mean	12.31	16.47	0.81	1.95
F test	***	***	***	***

*** Significant at $P = 0.01$

[a] BLS incidence = percentage of seedlings with bacterial leaf spot symptoms. Mean followed by same letters in a column are not significantly different based on the SNK test at $P = 0.05$. Each value is a mean of 48 seedlings

Table 3 The effect of treatment with selected plant extracts from *Aloe vera*, *Coffea arabica*, *Glycyrrhiza uralensis* and *Yucca schidigera* on tomato seed germination, seedling vigor and dry weight of tomato plants

Treatment	Seed germination[a]			Vigor index (%)[b]	Dry mass (g)
	NS (%)	ABS (%)	DS (%)		
Control					
Sterile distilled water	90.50b	3.00a	6.50a	702.51b	0.16a
Copper sulphate	92.80ab	2.30a	5.00ab	707.60b	0.14a
Untreated seed	92.00ab	3.50a	4.50ab	718.06b	0.16a
Plant extract					
A. vera	94.80a	1.80a	3.50ab	749.39ab	0.14a
C. arabica	95.50a	2.50a	2.00b	784.77a	0.14a
G. uralensis	92.80ab	2.80a	4.50ab	714.33b	0.16a
Y. schidigera	94.30a	2.00a	3.80ab	741.91ab	0.14a
Mean	93.20	2.50	4.30	731.22	0.14
F test	**	ns	**	**	ns

ns not significant

** Significant at $P = 0.05$

[a] Seed germination states: *NS* normal seedlings, *ABS* abnormal seedlings, *DS* dead seed. Each value is the percentage from 400 seeds test

[b] Seedling vigor index (Vi) was calculated as Vi = (mean root length + mean shoot length) × (percentage germination) (Abdul-Baki and Anderson 1973); means followed by the same letters in a column are not significantly different based on the SNK test at $P = 0.05$

seedlings compared to seeds treated with sterile distilled water (negative control). The number of normal seedlings obtained from seeds treated with these extracts was not significantly different from those obtained from copper sulphate (positive control) and untreated tomato seeds (Table 3). The number of normal seedlings obtained with seeds treated with plant extracts of *G. uralensis* was not significantly different ($P < 0.05$) from seed treatments using plant extracts from *A. vera*, *C. arabica* and *Y. schidigera*, (positive and negative controls, respectively). The number of abnormal seedlings was not significantly different ($P < 0.05$) between different seed treatments (Table 3). When the number of dead tomato seeds was compared between seed treatments, there was no significant difference ($P < 0.05$) between most treatments, except for tomato seeds treated with *C. arabica* (Table 3), which significantly increased ($P < 0.05$) tomato seedling vigor. The treatment of tomato seeds with extracts from *A. vera* and *Y. schidigera* were not significantly different ($P < 0.05$) from seeds treated with *C. arabica*, positive and negative controls (Table 3).

Seed treatment of tomato with plant extracts from *A. vera*, *C. arabica* and *Y. schidigera* significantly reduced the incidence and severity of BLS ($P < 0.001$) in all three experiments. Such effects were similar to those obtained for seedlings grown from tomato seeds treated with copper sulphate and untreated seeds (disease-free) control (Table 4). In all three experiments, the incidence and severity of BLS disease were significantly higher ($P < 0.001$) in tomato transplants of cultivars Tanya, Cal-J and Moneymaker treated with sterile distilled water (negative control) compared to the other treatments (Table 4).

Discussion

Tomato seeds were treated with aqueous extracts from 84 different plant materials to assess control of seed-borne infection of BLS of tomato caused by *X. perforans*. In the in vitro assays, 20.2 % of the tested plant extracts totally inhibited growth of *X. perforans* when seed washings from treated seeds were plated on NA. In the in planta experiments, notably 17.8 % of the tested plant extracts reduced BLS incidence by 100 % in tomato seedlings (Table 1). The most effective seed treatments, giving 100 % control in vitro and in planta, were obtained when tomato seeds were treated with plant extracts from *A. vera*, *B. pendula*, *C. arabica*, *G. uralensis*, *J. communis*, *O. basilicum*, *Q. robur*, *R. palmatum*, *R. officinalis*, *R. graveolens*, *S. alba*, *Y. schidigera* and *S. officinalis* (Table 1).

From the in planta evaluation of the 13 best performing plant extracts (Table 2), extracts from *A. vera*, *C. arabica*, *G. uralensis* and *Y. schidigera* were the most effective and promising for control of BLS of tomato when applied as seed treatment (Table 2). Such results indicate that these plant extracts have bactericidal properties and can be used for tomato seed treatment to control xanthomonads associated with BLS. Many reports are available on the antibacterial properties of these plants. The antibacterial activity of *A. vera* against *Shigella flexneri* and *Streptococcus*

Table 4 The effect of selected plant extracts from *Aloe vera*, *Coffea arabica* and *Yucca schidigera* applied as seed treatment on incidence and severity of bacterial leaf spot (BLS) caused by *Xanthomonas perforans* on three tomato cultivars under screenhouse conditions in Morogoro, Tanzania

Cultivar	Treatment	Experiment 1		Experiment 2		Experiment 3	
		Incidence (%)[a]	Severity index[b]	Incidence (%)	Severity index	Incidence (%)	Severity index
Tanya	Control						
	Sterile distilled water	45.00a	2.15a	82.50a	2.56a	65.00	2.18a
	Copper sulphate	10.00b	1.10b	0.00b	1.00b	2.50b	1.02b
	Untreated seed	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Extract						
	A. vera	2.50b	1.02b	0.00b	1.00b	0.00b	1.00b
	C. arabica	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Y. schidigera	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Mean	9.58	1.21	13.75	1.26	11.25	1.20
	F test	***	***	***	***	***	***
Cal-J	Control						
	Sterile distilled water	70.00a	2.18a	80.00a	2.55a	60.00a	1.65a
	Copper sulphate	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Untreated seed	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Extract						
	A. vera	2.50b	1.02b	0.00b	1.00b	2.50b	1.02b
	C. arabica	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Y. schidigera	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Mean	12.08	1.20	13.33	1.26	10.42	1.13
	F test	***	***	***	***	***	***
Moneymaker	Control						
	Sterile distilled water	65.00a	2.28a	67.50a	2.40a	72.50a	2.00a
	Copper sulphate	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Untreated seed	0.00b	1.00b	0.00b	1.00b	0.00b	1.00b
	Plant extract						
	A. vera	0.00b	1.00b	0.00b	1.00b	2.50b	1.02b
	C. arabica	2.50b	1.02b	0.00b	1.00b	0.00b	1.00b
	Y. schidigera	0.00b	1.00b	2.50b	1.02b	0.00b	1.00b
	Mean	11.25	1.22	11.67	1.23	12.50	1.17
	F test	***	***	***	***	***	***

*** Significant at $P = 0.01$b

[a] Percentage of seedlings with bacterial leaf spot symptoms

[b] Disease severity index based on Horsfall and Barrett (1945) scale of 1–6 with modifications: 1 = no disease and 6 = >50 % of leaves with BLS symptoms. Means followed by the same letters in a column are not significantly different based on SNK test at $P = 0.05$

pyogenes has been documented (Ferro et al. 2003). Extracts from *A. vera* also affect several Gram-positive and negative bacteria (Cock 2008). The antibacterial activity of extracts from *A. vera* was reported to be due to a direct effect on the bacterial cells caused by the presence of anthraquinones (Boateng 2000) and saponin (Reynolds and Dweck 1999; Urch 1999). Other indirect effects have been reported to be associated with the presence of polysaccharides, which stimulate plant defence responses that destroy bacteria (Lawless and Allan 2000; Pugh et al. 2001). Murthy and Manonmani (2009) reported that plant extracts from processed coffee inhibited growth of food-borne pathogens such as *Escherichia coli*, *Yersinia* and *Listeria* species. The antibacterial activity of coffee is associated with substances produced by the roasting process such as millard products, carbohydrate caramelization and thermal composition products (Daglia et al. 1994). In addition, *Glycyrrhiza* species contain α-glycyrrhetinic acid and glycyrrhizin, which inhibit DNA replication and RNA and protein synthesis of microbes (Kim et al. 2002). Other *Glycyrrhiza* species, e.g. *G. glabra*, inhibit growth of some Gram-negative bacteria such as *Salmonella* spp., *Shigella* spp. and *E. coli* (Shirazi

et al. 2007). Extracts from *Y. schidigera* also have antibacterial activity, which was attributed to the presence of saponin, a compound found to inhibit microbial growth through hemolytic activity (Hassan et al. 2010). In the present study, observations of cell suspensions of *X. perforans* treated with extracts from *Y. schidigera* at 10 % concentration using confocal microscopy revealed permeabilization of bacterial cells (data not shown).

In addition to reducing BLS in tomato seedlings, plant extracts from *A. vera*, *C. arabica* and *Y. schidigera* significantly improved germination ($P < 0.05$) of tomato seedlings (Table 3). At the same time, the vigor and dry mass of tomato seedlings were not affected by the treatment of seeds with plant extracts from *A. vera*, *C. arabica*, *G. uralensis* and *Y. schidigera*, indicating that these extracts were not phytotoxic to tomato seeds and seedling development. Other seed treatments with natural compounds of plant origin to control plant pathogens have been reported to have no negative effects on plant growth, seed viability or food quality (Opara and Wokocha 2008). Based on data from the present study, extracts from *A. vera*, *C. arabica* and *Y. schidigera* were the most promising plant extracts against BLS and can therefore be used to treat tomato seed. Additionally, they fulfilled the requirements for bioactive chemicals (Hewett and Griffiths 1986) and have the advantages of low mammalian toxicity, minimal health hazards and the least environmental pollution (Amadioha 2003; Singh 1994).

Under screenhouse conditions in Tanzania, using three tomato cultivars, transplants obtained from seed treated with extracts from *A. vera*, *C. arabica* and *Y. schidigera* had significantly lower BLS incidence and severity than tomato seedlings from seed treated with sterile distilled water (Table 4). The ability of these plant extracts to control BLS in the three tomato cultivars grown in Tanzania without negatively affecting seedling growth indicated the potential of using these plant extracts as seed treatment against BLS pathogens.

We also demonstrated that aqueous extracts of 13 plant species (15.5 % of the tested extracts) had antimicrobial properties and inhibited the growth of *X. perforans* in in vitro and in *planta* assays. Plant extracts from *A. vera*, *C. arabica* and *Y. schidigera* were of particular interest as a control strategy to BLS of tomato because they consistently inhibited *X. perforans* in different experiments without negative effects on tomato seeds and seedlings. The ability of these plant extracts to control BLS xanthomonads in tomato also provides an alternative control approach against copper-resistant strains reported to be present in Tanzania (Shenge et al. 2007). These plants extracts must also be tested against BLS under farmers' conditions before they can be recommended for production of BLS free tomato transplants. More research is also needed to identify bioactive fractions from these plant extracts as well as their mechanisms of action.

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