Journal of Medicinal Plants Research Vol. 6(10), pp. 1891-1895, 16 March, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR11.1272
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Antibacterial activity of clove, cinnamon, and datura extracts against *Erwinia carotovora* subsp. *atroseptica* causative agent of black stem and soft rot on potato

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Accepted 1 February, 2012

This study was carried out to evaluate the antibacterial activity of clove (*Eugenia caryophylata*) hexane extract, cinnamon (*Cinnamomum zelanicum*), and datura (*Datura metel*) ethanol extracts against the phytopathogenic *Erwinia carotovora* subsp. *atroseptica* growth, causative agent of black stem and soft rot on potato, in both cultured media and on plants in pots under natural conditions. Concentrations of 0.2, 0.5, 1, 3 and 5% of each extract were subjected for preliminary antibacterial assays against *E. carotovora* subsp. *atroseptica* in nutrient agar by pour plate method. The results of *in vitro* antibacterial activity showed that hexane extract (essential oil) of clove at 0.2% exhibits the highest inhibitory effect against the pathogenic bacteria, 100% compared to 80 and 90% with ethanol extracts of cinnamon and datura at the same concentration. The complete inhibition of *E. carotovora* subsp. *atroseptica* growth by cinnamon and datura extracts was achieved at 0.5% concentration. Similar results of the antibacterial activity of the extracts on *E. carotovora* subsp. *atroseptica*, on the plant in the pots under natural conditions, were obtained. The greatest suppression of potato black stem and soft rot disease was obtained on plants grown from extract-treated tubers sown in bacteria contaminated soil as well as on plants grown from bacteria-contaminated tubers sown in extract-treated soil (zero infection), compared with untreated tubers in contaminated soil, or contaminated tuber in untreated soil. The application of extracts on potato plant foliage grown from bacteria-contaminated tubers or in bacteria-contaminated soil had no effect on disease incidence, but the application of the extracts on the foliage before bacterial contamination highly inhibited the disease incidence.

Key words: Antibacterial, clove, cinnamon, datura, *Erwinia carotovora*, black stem, soft rot, potato disease, plant extracts.

INTRODUCTION

Black stem and tuber soft rot disease caused by *Erwinia carotovora* subsp. *atroseptica* is an important disease of potato worldwide. Potato can be infected in the field as well as during storage (Kelman and Maher, 1984). Currently, the disease represents as a major problem for potato producers in both field and storage in Iraq. The infected potato plants were characterized by development of black areas on the stem, beginning from the tuber, followed by yellowing, leaf rolling upward leading finally to wilting and death of the infected plants. The affected tissue becomes soft and watery, turning slimy with foul smell (Perombelon et al., 1987; Al-Jeboory and Al-Ani, 2010). Contaminated tubers, infected plant debris, and contaminated soil, were reported to be the main sources of infection in the fields (Bain et al., 1990; Perombelon et al., 1995). Although, *E. carotovora* is usually present on the surface of tubers, they require a wound to invade the interior (Kalab, 2002).

Control measures for bacterial disease were mostly restricted, for a long time, to the use of antibiotics, mainly streptomycin, but many pathogens had developed resistance strains toward the action of these antibiotics (Jones and Schnabel, 2000; Vanneste and Voyle, 2001). Several copper based compounds as alternative to

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antibiotic were also used, but they revealed insufficient efficiency under field conditions as well as reduced plant growth (Gracia-Garza et al., 2002). In addition, the use of these compounds poses many problems for ecosystem and public health (Baysal and Zeller, 2005). So, the necessity of finding natural effective and innocuous compounds alternative to synthetics against plant pathogenic bacteria is of great interest. It was reported that the higher plants are characterized by their ability to produce large number of organic compounds of so-called secondary metabolites, and they possess antimicrobial activities (Castello et al., 2002). Polysaccharides from plants were used as antimicrobe in agriculture and environmental protection (Bravin et al., 2006).

Aqueous extracts of young shoots of pear *Pyrus* spp. were found to exhibit strong activity against *Erwinia amylovora* (Jin and Sato, 2002). Antimicrobial activity of allicin from garlic (*Allium sativum*) against plant pathogenic bacteria including *E. carotovora* was proven *in vitro* and in plants tissue (Curtis et al., 2004). Aqueous infusion and essential oil of clove was found to exhibit high activity against several G- bacteria *in vitro* (Saeed and Tariq, 2008). Essential oil and methanic extract of *Thymus vulgaris* have been shown to possess antibacterial activities against several pathogenic bacteria (Al-Bayati, 2008).

Extracts from leaves and twigs of cinnamon *Cinnamomum zelanicum* showed high antibacterial activity (Ooi et al., 2006). It was reported that extract from *Datura stramonium* exhibit an inhibition effects on some bacteria and fungi in the culture media (Iranbakhsh et al., 2010). Plant extracts showed high efficiency in reducing systemic resistance in plants against many phytopathogens in Iraq (Al-Ani et al., 2010; Diwan et al., 2011; Al-Ani et al., 2011a, b,c).

During the last years, the diseases have received considerable attention due to great losses caused to potato yields in Iraq. The objective of this study is to evaluate the efficiency of ethanolic extracts of *Datura metel* and *C. zelanicum* and hexane extract of *Eugenia caryophylata* against *E. carotovora* subsp. atroseptica growth and disease development.

MATERIALS AND METHODS

Bacterial strain

Samples of leaves, stem, and tubers of potato plant showing yellowing, leaf curling with black areas on the stem from different fields at Abu-Ghraib, Iraq, were collected. The samples were surface sterilized by immersion in 1% sodium hypochlorite for 3 min and rinsed in distilled water. A piece of each sample was homogenized in sterile distilled water and a small part of the extract was streaked on nutrient agar (NA) by a sterile loop in Petri dishes of 9 cm diameter. The plates were incubated at 25 ± 2°C for 72 h. A well isolated colony on NA was suspended in 0.9% NaCl and streaked on NA as before. This step was repeated twice and conserved on slant NA at 4°C until use. The bacteri al strain were identified as *E. caratovora* subsp. *atroseptica* as reported by Al-Jeboory and Al-Ani (2010).

Plant materials

Aerial parts of datura were collected locally from the fields of College of Agriculture, University of Baghdad, Iraq, and identified by National Herbarium, Ministry of Agriculture. Flowers of clove and bark of cinnamon were purchased from the local market. The plant parts were dried in an incubator at 60°C for two days and grinded in a blender.

Extraction protocol

Hexane was used to extract the essential oil from clove, and ethyl alcohol 96% was used to extract the effective compounds from datura and cinnamon. One hundred grams of each powder were separately mixed with 200 ml of each solvent in containers of 500 ml and subjected to agitation for 24 h. The extracts were then filtered through filter paper (Whattman No. 1) in Buchner funnel with vacuum. The filtrates were concentrated by rotary evaporator at 50°C and conserved at 4°C until use.

Assay of antibacterial activity in culture media

The antibacterial activity of the extracts was carried out using pour plate method on nutrient agar medium (Iranbakhsh et al., 2002). The extracts were added to the medium before solidification (45°C) at 0.2, 0.5, 1, 3, and 5%. The mixtures were shacked and poured in Petri dishes of 9 cm diameter (20 ml/plate). The medium was inoculated with 0.1 ml of *E. caratovora* subsp. *atroseptica* inoculums (10^8 CFU/ml) prepared from 24 h old culture as already described. The inoculum was spread uniformly over the culture medium using sterile cell spreader. Plates without extracts were inoculated by the bacteria served as control. Colonies numbers were determined after 48 h of incubation at 25 ± 2°C and the inhibition percentage was calculated by dividing the value (colonies number of control – colonies numbers of treatment) on (colonies number of control) the product number multiplied by 100.

Effect of the extracts on *E. carotovora* subsp *atroseptica* in the plants under natural conditions

Treatments with extract

Potato tubers (Disirie) were soaked in clove extract for 15 min and in cinnamon and datura extracts for 30 min before sowing. The soil was treated by watering with the extracts (100 ml/pod); leaves and foliage of plants were sprayed with the extracts until run off, after germination.

Bacterial contamination

Potato tubers were soaked in *Erwinia carotovora* subsp. *atroseptica* suspension at 10^8 CFU/ml for 30 min before sowing. The soil in the pots was contaminated by watering with bacterial suspension during sowing.

Treatments

The tubers were sown in mix sterile soil (autoclaved at 121°C and 1.5 kg/cm^2 for 30 min for two successive days) in pots of 30 cm diameter. The pots were assigned to four groups. Pots of the first group were sown with tubers treated with extracts, in bacteria-contaminated soil. Pots of the second group were sown with tubers contaminated with bacteria in extracts-treated soil; while pots of the

Table 1. Antibacterial activity of different concentrations of clove *Eugenia caryophylata*, datura *Datura metel*, and cinnamon *Cinnamomum zelanicum*, on *Erwinia carotovora* subsp. *atroseptica* growth in culture media.

Concentration (%)	Clove extract		Datura extract		Cinnamon extract	
	Mean of colony number	Inhibition (%)	Mean of colony number	Inhibition (%)	Mean of colony number	Inhibition (%)
0.2	0	100	20	90	40	80
0.5	0	100	0	100	0	100
1	0	100	0	100	0	100
3	0	100	0	100	0	100
5	0	100	0	100	0	100
Control	200	0	200	0	200	0

third group were sown with bacteria-contaminated tubers in non-treated soil and the foliage of plants were sprayed with extracts after germination. The fourth group included non-treated/non-contaminated tubers sown in non-treated/non-contaminated soil, the foliage sprayed with extracts after germination then the soil watered with bacterial suspension after 48 h of extract application. Tubers were contaminated with bacteria and sown in non-treated soil, and these served as control.

The treatments were arranged in three replicates of 10 plants. Bacterial growth was recovered from potato plants by removing samples of stem and tubers. The samples were surface sterilized with 1% sodium hypochlorite for 3 min, washed in sterile water and homogenized in distilled water (1:1). Aliquots of the homogenate were spread on nutrient agar plates and incubated at 25 ± 2℃ for 48 h.

RESULTS

Antibacterial activity of the extracts in culture media

The results of the antibacterial activity of the plant extracts from, clove, cinnamon and datura against *E. carotovora* subsp. *atroseptica* in culture media are shown in Table 1. The data indicated that the essential oil of clove at 0.2% was more effective than ethanolic extracts of cinnamon and datura at the same concentration. The formation of colony on the medium containing 0.2% of clove extract was completely inhibited. While the inhibition percentage of cinnamon and datura extracts at 0.2% were 80 and 90%, respectively. The complete inhibition of *E. carotovora* subsp. *atroseptica* growth by Cinnamon and Datura extracts was achieved at 0.5%. At this concentration, no colony was formed on nutrient agar plates inoculated with the bacteria.

Inhibition activity of the extracts under natural conditions

Results of antibacterial activity of clove, cinnamon, and datura extracts on *E. carotovora* subsp. *atroseptica*, causative agent of potato soft rot were summarized in Table 2. Data showed that the three extracts exhibited antibacterial activity against the bacteria, as well as no bacterial growth was detected on nutrient agar plate inoculated by extracts from treated plants.

The greatest suppression of disease incidence was obtained on plants grown from tubers treated with extracts in bacteria-contaminated soil as well as from bacteria-contaminated tubers in extract-treated soil (zero infection); compared with untreated tubers in contaminated soil (100% infection).

Results also showed that the application of extracts on potato plant foliage, grown from bacterial contaminated tubers or in bacterial contaminated soil, had no effect on disease incidence. On the other hand, the application of extracts on the foliage before bacterial contamination highly inhibited the disease incidence.

DISCUSSION

The results of this study revealed clearly that the essential oil of clove *Eugenia caryophylata*, ethanol extracts of Cinnamon *C. zelanicum*, and Datura *Datura metel*, have the potential to restrict *E. carotovora* subsp. *atroseptica* growth in both the culture media and on plants under natural conditions. The antibacterial activity of cloves and cinnamon may be due to its contents of active component, euginol and cinnamic aldehyde (Beuchat and Golden, 1989), and alkaloid compounds (Wannang et al., 2009) in Datura, which may interact with cysteinyl residue of protein and other active groups leading to inhibiting of bacterial growth, disrupting of cell membrane and cell collapse. It was reported that euginol and cinnamic aldehyde extracted from clove and cinnamon, exhibit high activity against broad spectrum of bacteria (Moleyer and Narasimhan, 1992; Cowan, 1999; Alexander and Richard, 2004; Abu-Shanab et al., 2004; Yeh et al., 2009). The compound 5,7-dimethyl-6-hydroxyl, obtained from *D. metel* extract, was found to exhibit anti-bacterial activity against several G- and G+ bacteria (Okwu and Igara, 2009).

The most effective use of the extracts was found prior to post inoculation with bacteria. The greatest suppression

Table 2. Effect of clove, datura, and cinnamon extracts on *Erwinia carotovora* subsp. *atroseptica* on plants in pots under natural conditions.

S/N	Treatments		Bacterial growth on nutrient agar	Infection (%)
1	Treated tubers in contaminated soil	0.2% Clove extract	−	0
		0.5% Cinnamon extract	−	0
		0.5% Datura extract	−	0
2	Treated tubers in non-contaminated soil	0.2% Clove extract	−	0
		0.5% Cinnamon extract	−	0
		0.5% Datura extract	−	0
3	Contaminated tubers in treated soil	0.2% Clove extract	−	0
		0.5% Cinnamon extract	−	0
		0.5% Datura extract	−	0
4	Contaminated tubers in non-treated soil		+	100
5	Contaminated tubers in non-treated soil, sprayed the foliage by the extract after germination	0.2% Clove extract	+	80
		0.5% Cinnamon extract	+	80
		0.5% Datura extract	+	80
6	Non-treated/non-contaminated tubers in non-treated/non-contaminated soil, sprayed the foliage by the extract after germination and contaminated the soil, 48 h after spraying	0.2% Clove extract	+	20
		0.5% Cinnamon extract	+	20
		0.5% Datura extract	+	20

of disease incidence was obtained when tubers or soil were treated by the extract before sowing. This may be due to the reduction of initial inoculum concentration by the direct effect of active components in the extract against the bacteria. These results are in accordance with previous studies reporting that the main sources of primary inoculum reside in plant debris in the soil or coming with contaminated tubers (Bain et al*.,* 1990; Perombelon et al*.,* 1995). So, the control measures should focused on the reduction of primary source of infection by pre-treating the tubers and the soil with the extracts. When the infection was established, the curative application of extracts was ineffective as proven by disease incidence of 80%, when the foliage of plants was sprayed with the extracts after bacterial contamination presumably because externally applied extracts has lesser access to the bacteria. None of the aquatic extracts showed inhibition effects on bacterial growth (results not shown), this may be that the effective compounds of the extracts does not dissolve in water. There is a possibility that certain components of the extracts may induce systemic resistance in the plant is not excluded, as proven by a reduction of disease incidence to 20% when the foliage of plants was sprayed by the extracts 48 h before soil contamination with the bacteria. It was reported that plants possess defense mechanisms against pathogen attacks and some of these mechanisms are inducible by biotic and non-biotic agents (Walters et al., 2005). The resistance induced is characterized by restriction of pathogen growth and suppression of disease symptoms development. Due to increasing interest to use plant extracts for the preservation of food as antimicrobial agents (Smide and Gerris, 1999), and in medicine as therapeutic against various diseases in many development countries (Wanning et al., 2009), and against several phytopathogenic bacteria (Jin and Sato, 2003; Bysal and Zeller, 2004; Curtis et al., 2004; Bakht et al., 2011), the use of plant extracts in plant disease management, as alternative to synthetic chemicals may be promising.

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