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Antibacterial effect of *Chromolaena odorata* extracts on some *Erwinia* isolates, causal agents of tomato fruit rot in Dschang, Cameroon

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Abstract

Background: In spite of the fact that tomato fruits do not only serve as food but as medicine, nutrient supplement, flavouring ingredient, detoxificant and human system cleanser, the household consumption is on a constant increase worldwide. The microbial deterioration of tomato fruits causes reduction in market value and nutritional quality and at times renders the fruits unfit for consumption. This study was carried out to determine the in vitro and in vivo antibacterial effects of *Chromolaena odorata* leaves extracts on some *Erwinia* isolates, agent of post-harvest decay of tomato fruits.

Results: The results showed that the in vitro test of aqueous and ethanol extracts more significantly inhibited the growth of the two *Erwinia* isolates to the respective concentrations of 3 and 9 mg/ml with inhibition zones attaining 30 mm. The in vivo test revealed that these extracts have efficiency against bacterial isolates growth on the Cobra variety with 100% inhibition of EJD16 isolate for the ethanol extract. Conservative effect assessment revealed that the two extracts showed best preserved fruits of Cobra variety more than those of Rio Grande variety with a low rate of tomato fruit rot.

Conclusion: Based on the results obtained, the ethanol extract was more effective than the aqueous extract and completely inhibited the growth of EJD16 isolate on tomato fruits. Ethanolic extract of *C. odorata* could be recommended to extend the life span of tomato fruits.

Keywords: Tomato fruits, Rot, *Erwinia* isolates, *Chromolaena odorata*, Antibacterial effect

Background

Tomato (*Solanum lycopersicum* L.) is the third most cultivated and widely grown vegetable crop in the world. It is one of the most popularly produced and extensively consumed vegetable crops in the world [1]. The household consumption is a constant increase worldwide. It can be eaten raw in salads or as an ingredient in many dishes, and in drinks [2]. Naturally, it is very rich in vitamins, minerals, dietary fibre and protein [3]. Tomato fruits are not only food but also medicine, nutrient supplement, flavouring ingredient, detoxificant and human system cleanser [4]. Tomatoes and tomato-based foods provide a wide variety of nutrients and many health-related benefits to the body. Its production accounts for about 4.8 million hectares of harvested land area globally with an estimated production of 162 million tons [5].

In Cameroon, tomato fruits are available throughout the year and the average yield is 12 tons per hectare [6], with the West Region which is part of the major production areas. This production is threatened by some microbial fruits infection. In fact, microbial fruit infections often occur during crop cultivation, harvesting, post-harvest handling at processing, storage, transportation, packaging and distribution (loading and offloading) at

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various channels and selling outlets of which bacteria and fungi are prevalent [7–9].

The microbial deterioration of tomato fruits causes reduction in market value and nutritional qualities and at times renders fruits unfit for consumption. Tomato fruit being succulent with about 80% water content, low pH, rich nutrients elements and sugars constitutes a suitable medium for microbial growth [10, 11].

In Dschang locality (West Region of Cameroon), the most important constraint in tomato production, perceived by famers, is damage due to diseases (48%), followed by uncertainty of market (18%). Some of diseases observed in the fields cause severe losses of fruits in the field and during storage [12]. Research has also revealed that post-harvest loss of fruits due to microbial infections in Nigeria ranges between 50 and 90% [13, 14]. Post-harvest diseases cause economic losses in the field because of added costs of harvesting, transportation and storage [15]. Approximately 25–38% of harvested fruits and vegetables, respectively, are lost due to post-harvest spoilage in the U.S.A. and world markets [16].

There is therefore need to isolate and identify microorganisms associated with tomato fruits spoilage with the view to proposing suitable solutions of controlling them before they reach the final consumers, to safeguard human health. Public concern about fungicide residues on raw fruits and vegetables has stimulated research efforts using natural products to reduce incidence of post-harvest diseases. The objectives of this study therefore focused, firstly, on isolation and identification of pathogenic microorganisms associated with tomato fruit spoilage in Dschang (West Region, Cameroon) and, secondly, to evaluate natural products for biopesticidal activity against pathogenic bacteria associated with tomato post-harvest diseases.

Methods

Collection of tomato fruits

Infected and uninfected tomato fruits were collected from farmers as well as retailers in some localities and markets of Dschang in June, July and August 2016. A physical examination by visual observation of fruits determined the selection of the fruits to be considered in the sample. Samples of infected and uninfected fruits were transported separately in sterile polyethylene bags to the laboratory of Phytopathology and Agricultural Zoology. In the Laboratory, infected fruits were used for isolation of bacteria and uninfected fruits were used for pathogenicity and in vivo tests.

Plant extracts

Aerial parts (leaves and stems) of *Chromolaena odorata* were collected in July 2016 from Dschang. Their identification was confirmed through consultation in the Herbarium of the Department of Plant Biology, University of Dschang. Plant parts collected were washed three times with running tap water and rinsed with sterile distilled water. They were separately air-dried at room temperature and ground in a mortar. One hundred grams of the final dried powder was macerated in 500 ml of distilled water or ethanol and mixed thoroughly. For aqueous extract, the mixture was allowed to rest for 48 h and the supernatant was passed through a Whatman No. 1 filter paper to obtain the extract. For ethanolic extract, after maceration for 4 h in a warring blender (Warring International, New Hartford, CT, USA), the macerate was passed through a Whatman No. 1 filter paper and evaporated using a Rota vapour at 40 °C water bath temperature (Heidolph) [17]. Extracts were preserved aseptically in a brown bottle at 4 °C until further use [18].

Bacterial strains

Two bacterial isolates belonging to the genus *Erwinia* (EBD16 and EJD16) provided by the Laboratory of Plant Pathology and Agricultural Zoology of the University of Dschang were used for the in vivo tests. These microorganisms were isolated from infected tomato fruits collected from farmers as well as retailers. The species of the genus *Erwinia* were chosen because some species of *Erwinia* are responsible for rotting of tomato fruits in the field and sometimes after harvest.

Pathogenicity tests

Pathogenicity tests were performed on bacterial isolates by using the fruits of two tomato cultivars (Rio grande and Cobra) as previously described by [19] and taken up by [20] with a few modifications. Uniform fruits based on size and colour, free from wounds and showing no symptoms of disease were selected. They were washed with tap water, surface-sterilized by dipping in 1% sodium hypochlorite solution for 5 min, rinsed by dipping three times in sterile distilled water for at least 10 min, and dried on blotting paper. A wound (5 mm diameter by 1 mm depth) was made on each fruit using a pipette tip.

Fruits were inoculated with 100 µl of a bacterial suspension (1×10^8/CFU). After fruits inoculation, wounds were covered with tape. Inoculated fruits were placed in a plastic box containing sterile paper towels moistened with sterile water and incubated for 72 h at. An organism was recorded as pathogenic if symptoms of rot appeared on the tested fruit. The experiments were set up with four replications, and each experiment was repeated twice. Control fruits have subjected to the same operations with the only difference that they were inoculated by physiological water. Virulence of each isolate was assessed by measuring the lesions surface of inoculated fruit caused by these isolates after incubation at 21 ± 2 °C for 72 h.

Assays of in vitro antibacterial activity

Antibacterial activity of aqueous and ethanolic extracts of *C. odorata* was determined by disc diffusion method on PCA medium. Sterile Whatman filter discs (6 mm diameter) were made in PCA plate using sterile cork borer (5 mm), and inoculum containing 10^8 CFU/ml of bacteria was spread on the solid plates with a sterile swab moistened with the bacterial suspension. Then, 100 µl of each of all aqueous and ethanolic extracts (concentrations of 1, 2, 3 and 7, 8, 9 mg/ml, respectively) was placed in the discs made in inoculated plates. The treatments also included 100 µl of solvents (distilled water and ethanol) as negative control and fungicide as standard control (as locally used by producers). The plates were incubated for 48 h at 37 °C, and zone of inhibition if any around the discs was measured in mm (millimetre). Each treatment consisted of three replicates and repeated at least twice.

In vivo antibacterial activity

Fruits apparently healthy were selected based on uniformity in size and colour, and free from wounds. They were washed with tap water, surface-sterilized by dipping in 1% sodium hypochlorite solution for 5 min, rinsed by dipping three times in sterile distilled water for at least 10 min, and dried on blotting paper. A wound (5 mm diameter in 1 mm deep) was made on each fruit using a pipette tip.

The fresh fruits were inoculated with 100 µl of a bacterial suspension (1×10^8/CFU) and each received 100 µl of extract solutions extracts aqueous and ethanolic at concentrations 3 and 9 mg/ml, respectively. Inoculated and treated fruits were placed in a plastic box containing sterile paper towels moistened with sterile water and incubated for 72 h at 21 ± 2 °C (ambient laboratory temperature). The treatments also included 100 µl of solvents (distilled water and ethanol) served as negative control and fungicide as standard control. After 72 h, lesions on fruit surfaces were measured.

Protective effect of the extracts on the conservation of tomato fruits

Fruits apparently healthy were selected based on uniformity in size and colour, and free from wounds. Fruits were washed with tap water, surface-sterilized by dipping in 1% sodium hypochlorite solution for 5 min, rinsed by dipping three times in sterile distilled water for at least 10 min, and dried on blotting paper. Then fruits were soaked for 5 min in *C. odorata* solutions at concentrations 3 and 9 mg/ml, respectively, for aqueous and ethanol extracts and air-dried in the laboratory. Treated and untreated (negative control) fruits were placed in a plastic box containing sterile paper towels moistened with sterile water and stored at 21 ± 2 °C (ambient laboratory temperature) for 15 days. After 15 days of storage, the number of fruits with the rots was counted for each treatment.

Statistical analysis

Data on bacterial lesions, inhibition halos and the number of fruits damaged during the storage were submitted to analysis of variance. Means were separated by the Duncan's test at 5% probability threshold.

Results

Pathogenicity of *Erwinia* isolates

Tomato fruits inoculated with bacterial isolates all developed rot. *Erwinia* isolates were associated with the maceration observed on the fruits. Indeed, the test was performed on the apparently healthy fruits showing no symptoms of disease or visible lesions. Nevertheless, some fruits of control developed rot, although, less important than the damage caused by *Erwinia* isolates. The rot surfaces caused by *Erwinia* isolates (EJD16 and EBD16) were not significantly different for the Grande Rio variety (Table 1). For the Cobra variety, EJD16 showed the largest damage with rot surfaces of 644.33 mm^2 after 72 h of incubation.

In vitro effect of *C. odorata* extracts on bacterial growth

Table 2 shows in vitro effects of aqueous and ethanol extracts of *Chromolaena odorata* on two bacterial isolates. The results showed that, overall, aqueous and ethanol extracts had an inhibitory effect on the two bacterial isolates compared to the negative control. However, this inhibitory effect varied with concentrations, bacterial strains and the type of extract.

There was no significant difference ($P > 0.05$) between inhibition halos obtained at concentrations of 1, 2 and 3 g/ml of aqueous extract on the isolate EBD16. For isolate EJD16, the concentration of 3 g/ml of the aqueous extract was the most inhibitory with an average diameter of inhibition of 30.07 mm. All the three concentrations of ethanol extract tested proved to be effective on the EJD16 isolate with inhibition halos ranging from 22.51

Table 1 Surfaces of bacterial rot on fruits after 72 h

Tomato variety	Isolate	Lesion (mm^2)
	Control	119.00 ± 35.68^{b}
Rio Grande	EBD16	386.33 ± 87.87^{a}
	EJD16	412.33 ± 116.64^{a}
	Control	$113.33 \pm 89.90^{c*}$
Cobra	EBD16	413.33 ± 48.79^{b}
	EJD16	644.33 ± 148.86^{a}

Means followed by the same alphabetical letter in the same column are not significantly different according to Duncan's test at $P \leq 0.05$

Table 2 Inhibition halo (mm) of the bacterial isolates by extracts of *C. odorata*

Treatment	Erwinia isolates	
	EJD16	EBD16
Aqueous extract		
T−	0.00 ± 0.00^{d}	0.00 ± 0.00^{c}
T+	39.87 ± 3.35^{a}	36.97 ± 3.40^{a}
1 g/ml	25.73 ± 0.23^{c}	27.87 ± 1.97^{b}
2 g/ml	24.30 ± 3.48^{c}	25.73 ± 0.51^{b}
3 g/ml	30.07 ± 0.80^{b}	27.63 ± 0.35^{b}
Ethanolic extract		
T−	10.97 ± 0.91^{c}	13.20 ± 0.72^{d}
T+	39.87 ± 3.35^{a}	36.97 ± 3.40^{a}
7 mg/ml	23.50 ± 2.85^{b}	20.53 ± 3.93^{c}
8 mg/ml	22.50 ± 1.15^{b}	23.40 ± 1.31^{bc}
9 mg/ml	25.16 ± 0.51^{b}	27.97 ± 3.25^{b}

T − = negative control; T + = positive control

Means affected with the same alphabetical letter in the same column are not significantly different according to Duncan's test at $P \leq 0.05$

to 25 mm. Against the EBD16 isolate, ethanolic extract was more active at the concentration of 9 mg/ml with an average inhibition diameter of 27.97 mm. It should be noted that the positive control showed the larger halos of inhibition as on the EJD16 isolate and against EBD16 isolate with halos of 39.87 and 36.97 mm, respectively.

Effect of *C. odorata* extracts on bacterial growth on tomato fruits

Results of the antibacterial tests on the tomato fruits inoculated with bacterial isolates are reported in Table 3. It appears from these results that effect of *C. odorata* extracts varied depending on the strains and tomato varieties. Different treatments applied on the Rio Grande variety tomato fruits showed no significant difference between the two bacterial isolates and compared to the negative control. On the Cobra variety, the ethanol extract showed an inhibitory effect statistically different from other treatments. Against the EJD16 isolate, inhibition was complete with the ethanol extract. Regarding the EBD16 isolate, the ethanol extract showed the greatest inhibition with 83 mm^2 of lesion which was statistically very lower than those of other treatments.

Protective effect of *C. odorata* extracts on the tomato fruits in conservation

The test results of tomatoes fruits conservation with *C. odorata* extracts are grouped in Table 4. It appears from these results that extracts decreased the number of rotten fruits depending on the variety. For this purpose, the number of rot fruit of Rio Grande variety was significantly low compared to that of the negative control contrary to Cobra variety where extracts had no effect on the number of rotten fruit. The two extracts showed best preserved fruits of Rio Grande variety than Cobra variety. Also the positive control and the ethanol extract showed the greatest conservative effect on the fruits of Rio variety.

Discussion

Tomato fruits inoculated with bacterial isolates all developed rot, which reflects the fact that the *Erwinia* isolates used were associated with the maceration observed on the fruits. But some fruits of control developed rot, however, less important than the damage caused by *Erwinia* isolates. This could be due to an

Table 4 Average number of rotten tomato fruits by variety after storage

Treatment	Rio Grande	Cobra
T−	3.66 ± 1.15^{a}	2.66 ± 2.08^{ab}
T+	1.00 ± 1.73^{b}	1.00 ± 1.73^{ab}
Aqueous	3.33 ± 0.58^{ab}	3.00 ± 0.00^{a}
Ethanolic	1.33 ± 1.52^{ab}	0.66 ± 1.15^{b}

T − = negative control; T + = positive control

Means followed by the same alphabetical letter in the same column are not significantly different according to Duncan's test at $P \leq 0.05$

Table 3 Surface of bacterial lesions on treated and untreated tomato fruits

Treatment	Lesions surface (mm^2)			
	Rio Grande		Cobra	
	EJD16	EBD16	EJD16	EBD16
T−	481.00 ± 71.42^{a}	387.33 ± 88.07^{a}	585.00 ± 201.43^{a}	395.67 ± 70.68^{a}
T+	402.33 ± 452.36^{a}	361.00 ± 112.52^{a}	162.33 ± 5.85^{b}	535.00 ± 360.45^{a}
Aqueous	350.00 ± 35.36^{a}	328.33 ± 80.82^{a}	180.00 ± 10.00^{b}	222.00 ± 30.11^{ab}
Ethanolic	152.33 ± 137.42^{a}	312.00 ± 187.50^{a}	0.00 ± 0.00^{c}	83.33 ± 87.80^{b}

T − = negative control; T + = positive control

Means followed by the same alphabetical letter in the same column are not significantly different according to Duncan's test at $P \leq 0.05$

infection through micro-injuries caused by the poor conditions of handling during the transportation of the fruits from production field to the market places [21, 22]. The results obtained in this study are in agreement with those of several authors who demonstrated that bacterial isolates of the genus *Erwinia* cause the maceration of the fruits of tomato, cabbage, carrot and potato [20, 23, 24].

Antibacterial test results revealed that aqueous and ethanol extracts of *C. odorata* had an inhibitory effect against the two bacterial isolates compared with the negative control. Nevertheless, this inhibitory effect varies with the concentrations, the type of extract and bacterial isolate. These results are similar to those showing that methanol extracts from *Chromolaena odorata* leaves have an inhibitory effect on *Xanthomonads vesicatoria* and *Ralstonia solanaccearum* with inhibition zones of 12 mm [25].

It is also reported that the aqueous and methanolic extracts of the bark and roots of *Chromolaena odorata* have a significant inhibitory effect on human pathogenic bacteria [26]. In the same logic, some previous work has shown that methanol extracts of the leaves of the same plant present the largest inhibition zone (19 mm) against *Vibrio harveyi* [27].

These antibacterial properties might be due to the presence of phenolic compounds and flavonoids contained in this plant [28]. These compounds spread into the bacterial membrane, damage it and cause the death of the cell [28].

Ethanol and aqueous extracts of *C. odorata* were best preserved fruits of the variety Rio Grande compared to those of Cobra variety. This result could be justified by the fact that the post-harvest treatments of fruits such as the coating have no effect on pathogens when infection is prior to treatment. Once the pathogen has won the fruit inside, the surfaces sterilization and other surface treatment cannot have an effect [29, 30].

Conclusion

Results of this study revealed that *Erwinia* isolates used were associated with the maceration observed on the tomato fruits. Also, the ethanol extract of *C. odorata* was more effective than the aqueous extract against *Erwinia* isolates. *In vivo* evaluation shows that the two extracts showed best preserved fruits of Cobra variety than the Rio Grande variety. Ethanol extract completely inhibited the growth of EJD16 isolate on tomato fruits. Ethanolic extract of *C. odorata* could be used to extend the life span of tomato fruits. It would be very interesting to continue the study on other pathogenic bacteria.

Authors' contributions

YA conceived of, but all authors planned the study. NKE led the work in the laboratory, ZMAS followed the work in the laboratory, and EELC prepared data and performed statistical analysis, under the guidance of YA. All authors drafted the manuscript, critically reviewed it for important intellectual content and contributed to the interpretation of results. All authors read and approved the final manuscript.

Authors' information

Yaouba Aoudou received his Ph.D. in Microbiology and Food Biotechnology from the National High School of Agro-Industrial Sciences, Cameroon. His research interests are includes the assessment of the impact of microbial infection of crops in field and in storage, and the search for alternatives to fight against these microorganisms by the use of natural substances of plant origin. NNOMO Ketsia Emilienne, an MSc student in Phytopathology, and Ze Medjap and Essola Etoa louis, Ph.D. students in Phytopathology, are all interested in the assessment of the impact of microbial infection of crops, and the search for alternatives to fight against these organisms by the use of natural substances of plant origin.

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Competing interests

The authors declare that they have no competing interests.

Availability of supporting data

The data analysed in this study are available from the corresponding author on request.

Consent for publication

All authors consent to the publication of this manuscript.

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