Acta Biológica Catarinense
2023 Jul-Set;10(3):4-10

Susceptibility of cassava accessions and microbial activity of plant extracts in the *in vitro* control of *Xanthomonas axonopodis* pv. *manihotis*

Susceptibilidade de acessos de mandioca e atividade microbiana de extratos vegetais no controle in vitro *a* Xanthomonas axonopodis *pv.* manihotis

Thainá Fogliatto **MOREIRA**[1, 3]; Juliane Nicolodi **CAMERA**[2]; Jana **KOEFENDER**[2]; André **SCHOFFEL**[2] & Diego Pascoal **GOLLE**[2]

ABSTRACT
In Brazil, the most important disease in cassava is bacteriosis, caused by *Xanthomonas axonopodis* pv. *manihotis*. The variety of substances present in plants has provided an increase in research using plant extracts for *in vitro* control of phytopathogens. This work aimed to evaluate different extracts for the control of *Xanthomonas axonopodis* pv. *manihotis* as well as to evaluate which accessions are susceptible to this bacteriosis. The work was divided into two experiments, one in a greenhouse and the other in the laboratory, both in a completely randomized design with five replications. The propolis, oregano and star anise extracts tested in this research proved to be a control option. Understanding the antimicrobial effect of compounds present in plant extracts may constitute yet another way of alternatively controlling pathogens in cultivated plants. Regarding the cassava accessions, the use of the Fepagro RS14 cultivar and the FV10, FV13 and SJ06 accessions is recommended, as these proved to be resistant to bacteriosis.
Keywords: alternative control; bacteriosis; genetic resistance.

RESUMO
No Brasil, a doença mais importante da mandioca é a bacteriose, causada por *Xanthomonas axonopodis* pv. *manihotis*. A variedade de substâncias presentes nas plantas tem proporcionado um aumento nas pesquisas utilizando extratos vegetais para o controle *in vitro* de fitopatógenos. Este trabalho teve como objetivo avaliar diferentes extratos para o controle de *Xanthomonas axonopodis* pv. *manihotis*, bem como avaliar quais acessos são susceptíveis a essa bacteriose. O trabalho foi dividido em dois experimentos, um em casa de vegetação e outro em laboratório, ambos em delineamento inteiramente casualizado com cinco repetições. Os extratos de própolis, orégano e anis-estrelado testados nesta pesquisa mostraram-se uma opção de controle. Compreender o efeito antimicrobiano de compostos presentes em extratos de plantas pode constituir mais uma forma alternativa de controlar patógenos em plantas cultivadas. Quanto aos acessos de mandioca, recomenda-se o uso da cultivar Fepagro RS14 e dos acessos FV10, FV13 e SJ06, pois estes se mostraram resistentes à bacteriose.
Palavras-chave: bacteriose; controle alternativo; resistência genética.

Recebido em: 2 maio 2023
Aceito em: 3 ago. 2023

[1] Faculdade de Agronomia, Programa de Pós-Graduação em Fitotecnia, Av. Bento Gonçalves, n. 7712 – CEP 91540-000, Porto Alegre, RS, Brasil.

[2] Curso de Agronomia, Universidade de Cruz Alta (Unicruz), Cruz Alta, RS, Brasil.

[3] Corresponding author: thainafogliatto@gmail.com.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is known for its social role, especially among the poor in tropical regions. In addition, it is a low-cost, rustic culture, with excellent adaptability to several environments (ALVES, 2002). Elected as food of the 21st century by the United Nations for Food and Agriculture Organization (FAO), cassava may contribute to the reduction of hunger and rural poverty, due to its versatility and ease of cultivation (TIRONI *et al*., 2019).

Although the crop presents rusticity, it also becomes the target of pests and diseases that cause a reduction in crop productivity and may cause total loss (ALVES, 2002). According to Fonseca (2018), it is estimated that cassava is host to approximately 30 different agents, including fungi, bacteria, viruses or similar, in addition to phytoplasma (mycoplasmas). In Brazil, the disease that significantly affects cassava is bacteriosis, caused by *Xanthomonas axonopodis* pv. *manihotis*, whose symptoms include leaf spots and necrosis, wilt, death of descendant, gummy exudation and necrosis of the vascular system of the plant (ISHIDA *et al*., 2016). The Brazilian regions most affected by the disease are the South, Southeast and Central-Midwest, in which this bacteriosis can have a high destructive power, reducing the propagating material and the cultivation areas, due to the permanence of the inoculum (SOUZA & FIALHO, 2003). Due to the systemic mobility of the pathogen, there are no curative control options, however, it is recommended to use integrated methods such as cultural controlling, use of tolerant cultivars and actions aiming to avoid the insertion of highly virulent strains in areas of low or no infestation (HILLOCKS & WYDRA, 2002) being these the control methods that have been used so far, in Brazil, and there are no chemicals registered to control this disease.

The objective of this work was to identify sources of alternative control of bacteriosis, using aqueous extracts, which were tested *in vitro* and to evaluate cassava accessions for susceptibility to *Xanthomonas axonopodis* pv. *manihotis*.

MATERIAL AND METHODS

The experiments were carried out at the Laboratory of Medicinal Plants and Plant Diseases and at the experimental area of the Center of Technological Innovation of Alto Jacuí, both located at Cruz Alta University (Unicruz). Evaluation of the susceptibility of cassava cultivars to *Xanthomonas axonopodis* pv. *Manihotis.* For the execution of this experiment, the Xam P99 isolates, from Embrapa Amazônia Oriental de Belém, state of Para, Brazil, were used. The multiplication of the isolate was carried out in Medium Kado 523 by Kado & Heskett (1970), composed of 10g sucrose, 8g hydrolyzed acid casein, 4g yeast extract, 2g dibasic potassium phosphate, 0.3g magnesium sulfate, 15g agar, 1000 ml water distilled. After 48 hours of incubation, the culture was suspended in sterile distilled water and the concentration of bacterial cells was adjusted to $2x10^9$ c.f.u. (Colony Forming Unit). With this process, the bacterial suspension of inoculation of the cultivars was obtained.

The five accessions (FV10, FV13, SJ 06, CA 08, SJ 03) and a control cultivar (Fepagro RS14), used during the experiment, belong to the Cassava Germplasm Bank, of the University of Cruz Alta, State of Rio Grande do Sul, Brazil. Cassava seedlings were obtained through the rapid propagation method, which consists of inducing rooting of stem cuttings of two buds, for the production of herbaceous shoots, and their subsequent rooting (CEBALLOS *et al*., 1980; KOEFENDER *et al*., 2017). After the shoots reached a height of over 15 cm, they were cut with blades disinfested in 70% alcohol (v / v) and, immediately after cutting, the rooting phase was continued, which was conducted in a greenhouse. When they were out of risk of death, the seedlings uniformly selected, started to grow in plastic pots with a capacity of two liters, filled with the soil of the region.

The experiment was carried out in a completely randomized design, with five replications, and the experimental plot consisted of one plant *per* pot.

At 42 days after planting, when the plants had an average of 5 to 6 leaves, the pathogen was inoculated into the leaves and stems. The inoculation on the leaves was performed with the aid of small-sterilized scissors, which was previously soaked in the bacterial suspension of the isolates, so that this bacterium could enter in the plant. Cuts were made in the three central leaflets of

three fully open leaves, counted from top to bottom. After cutting each leaflet, the scissors were again soaked in the suspension. In the inoculation of the stem, wooden sticks (broken in half and sterilized) previously immersed in bacterial suspension, for 10 minutes, were used. The toothpicks were introduced carefully into the petiole insertion region of the oldest leaf, so that they did not cross the stem of the plant. After inoculating the leaves and stem, each plant was sprayed with water and covered with a moistened plastic bag. In this condition, the plants were kept in a greenhouse for 48 hours. After that period, the plastic bags were removed and the plants remained in a greenhouse condition.

The evaluation was carried out at 41 days after inoculation, to determine the severity of the disease in each plant. The use of the scale of grades developed by Aquiles (2014) was adopted. This scale establishes the intensity of the visible symptoms in the infected plant, assigning scores from 1 to 6 where: 1 = No symptoms of the disease; 2 = Partial recovery without descending death from the apex of the plant; 3 = Partial recovery with descending death from the apex of the plant; 4 = Presence of pus along the stem without or with partial recovery; 5 = Descending death of the plant, with the presence of pus and partial recovery; 6 = Descending death of the plant, with the presence of pus and without partial recovery.

IN VITRO EVALUATION OF MICROBIAL ACTIVITY OF THE PLANT EXTRACT

For the test, extracts of propolis, star anise (*Illicium verum* Hook.f.), marjoram (*Origanum majorana* L.), basil (*Ocimum basilicum* L.) and oregano (*Origanum vulgare* L.) were used. Plant material was obtained from local stores and propolis was used with a commercial extract of Wax Green 30%. To obtain the aqueous extracts, 20 g of the plant material was crushed in a blender, with 100 ml of distilled and sterilized water. For propolis, dilution with distilled water to a concentration of 20% was performed.

An aqueous suspension (0.1 ml) of *Xanthomonas axonopodis* pv. *manihots*. (10^8 cel / ml) with 48 hours of culture was spread with the aid of a glass handle over the surface of the Petri dishes containing the PDA (Potato Dextrose Agar) medium. After, the filter paper discs (15 mm Ø), sterilized in an autoclave, were soaked for one minute in the different plant extracts and equidistantly distributed over the Petri dish containing the bacterium suspension.

The plates were kept at a temperature of 30°C, for 48 hours, in a photoperiod oven (BOD – biochemical oxygen demand). After this period, the inhibition halos were measured with the aid of a caliper.

A completely randomized experimental design with five replications was used. Five extracts were at a concentration of 20%, compared to streptomycin as a negative control and a control without extracts.

RESULT ANALYSIS

Data were subjected to analysis of variance and the means were compared by the test of Tukey at 5% error probability by means of SISVAR software (FERREIRA, 2011).

RESULTS AND DISCUSSION

The results showed that the propolis extract inhibited 64.38% of bacterial growth. The extracts of star anise and oregano resulted in 34.93% and 30.82% inhibition, respectively, both of which did not differ statistically. The extract of marjoram inhibited 13.01% and basil did not differ from the control, with an inhibition of 0% (table 1).

Table 1 – Plant extracts correlated to the inhibition of bacteriosis by *Xanthomonas axonopodis* pv. *manihotis* in cassava crop.

Plant extract	Growth inhibition (%)
Negative control	100.00 a[1]
Propolis	64.38 b
Star anise	34.93 b
Oregano	30.82 c
Marjoram	13.01 d
Basil	0.00 e
Control	0.00 e
CV (%)	14.23

[1] Means followed by the same letter in the columns are not different from each other by the test of Tukey at 5% probability.

The use of alternatives such as plant extracts, in the control of plant diseases, has been used and discussed worldwide. It is known that extracts have demonstrated a positive effect on the control of phytopathogens in crops of great economic value (MARTINI, 2015).

Several studies have already been carried out using plant extracts to combat phytopathogens in plants and studies show that many species have become efficient in controlling pathogens, which is related to their antifungal and antimicrobial activity, operating directly or indirectly on the disease, triggering plant defense (STANGARLIN *et al*., 2010).

Propolis has become one of the options in the control of phytopathogens (PEREIRA, 2004), as it presents in its composition a complex mixture of substances, formed by resins and balms of plants of the local bee flora and of the beewax (SILVA, 2013). According to Bianchini & Bedendo (1998), propolis has bactericidal and/ or bacteriostatic action. For Baldin (2014), propolis showed a direct action on the development of bacteria of *Xanthomonas* genus. Gonçalves (2022), in his studies, found that extracts of Brazilian propolis (red (PBVM) and brown (PBMA)), are natural products with antibacterial and antibiofilm action against *Xanthomonas citrus*.

The microbial activity of propolis is mainly referred to flavone pinocembrine, phenylethyl ester of caffeic acid and flavonol galagine (UZEL *et al.,* 2005). These components are considered to act on the membrane or cell wall of the microorganism, causing structural and functional damage (SCAZZOCHIO *et al*., 2005). Schauffler (2017) found that propolis extract had an antimicrobial effect on the bacteria *Xanthomonas gardneri* and *Pseudomonas syringae*.

Silva (2018) reported that phytopathogenic bacteria, *Agrobacterium tumefaciens*, *Pseudomona syringae* and *Xanthomona axonopodis,* could be controlled by ethanolic or aqueous propolis extracts. These results are in line with those obtained in this work as propolis showed an efficient *in vitro* control of the bacteria under study.

Star anise and oregano extract also demonstrated significant antimicrobial action on the bacteria. According to Lima *et al*. (2008), the chemical composition of star anise is 90% trans-anethole, in addition to cis-anethole, methylchavicol and anisaldehyde, and its properties are mainly attributed to trans-anethole, which has antifungal, antimicrobial and antiseptic action. As for the oregano extract, it is possible to observe that it has developed the induction of the production of phytoalexins, personalizing eliciting activity (STANGARLIN *et al*., 2010). According to studies by Milos *et al*. (2000), oregano species have demonstrated antimicrobial and antioxidant action, however, these authors emphasize that their biological properties may vary according to the cultivation technique, origin, vegetative stage and the season of collection of the plant material.

Regarding the marjoram extract, there was a low inhibition of *Xanthomonas axonopodis* pv. *manihotis*. Such evidence may be related to the fact that this bacterium is a gram-negative and, according to Bagamboula *et al*. (2004), gram-negative bacteria are less susceptible to compounds found in plant extracts, due to the impediment of the compounds in propagating the outer membrane, originated by the existence of a hydrophilic barrier, which, although not totally impermeable, impairs the

passage of macromolecules and hydrophobic components. Due to their antifungal and antimicrobial properties, several spices have been mentioned in papers, such as marjoram (*Origanum majorana* L.) (CELIKTAS *et al*., 2007).

As for the action of plant extracts on the inhibition in the formation of colonies of the bacterium, it was noticed that propolis, star anise and oregano obtained the best results in comparison to marjoram and basil, which did not differ statistically in relation to control (table 2).

Table 2 – Inhibitory action of plant extracts on the formation of colonies of the bacterium *Xanthomonas axonopodis* pv. *manih*otis. Caption: CFU = colony forming unit.

Plant extracts	C.F.U. number
Negative control	0.00 a[1]
Propolis	4.25 b
Oregano	20.00 b
Star anise	38.50 c
Marjoram	1924.00 d
Basil	1944.25 d
Control	2011.25 e
CV(%)	8.4

[1] Means followed by the same letter in the columns are not different from each other by the test of Tukey at 5% probability.

The plant species used in this experiment may become useful in the alternative control to *Xanthomonas axonopodis* pv. *manihotis* because the raw material of the extracts is easy to be acquired and, with that, it is possible for the producer to carry out this method on the farm. To determine the analysis of the accession resistance, scores were assigned according to the visible symptoms. It was found that the accessions CA08 and SJ03 showed susceptibility to the presence of the bacteria. In contrast to SJ06, FV13 and FV10 proved to be resistant, and the cultivar Fepagro RS14 proved to be highly resistant (table 3).

Table 3 – Reaction of cassava accessions to bacteriosis caused by *Xanthomonas axonopodis* pv. *manihotis.*

Cultivars/ accessions	Cultivar reaction
Fepagro RS14	Highly resistant
FV10	Resistant
FV13	Resistant
SJ06	Resistant
CA08	Susceptible
SJ03	Susceptible

The high resistance shown by the cultivar Fepagro RS14 can be attributed to its phenological characteristics and because it is considered a forage cassava. According to Portz *et al*. (2009), table varieties have a higher incidence of *Xanthomonas axonopodis* pv. *manihotis* when compared to varieties for industry or forage, which corroborates with the result found in this experiment.

CONCLUSION

Vegetable extracts will be able to be used as an alternative in the control of phytopathogens, resulting in a more sustainable production.

The extracts of propolis, oregano and star anise tested in this experiment were found to be a control option, resulting in a viable alternative to the use of pesticides.

The cultivar Fepagro RS14 is highly resistant and the accessions FV10, FV13 and SJ06 are resistant to *Xanthomonas axonopodis* pv. *manihoti*.

ACKNOWLEDGEMENTS, FINANCIAL SUPPORT AND FULL DISCLOSURE

To Unicruz, Emater/RS and Embrapa. The authors declare that there is no conflict of interest in the publication of this paper.

REFERENCES

Alves, A. A. Cassava: biology, production and utilization. In: Hilocks, R. J., Tresh, J. M. & Bellotti, A. C. (Eds.). CABI international. E-book. 2002. p.67-89.
doi: doi/book/10.1079/9780851995243.0000

Aquiles, K. R. Propagação rápida de *Manihot esculenta* (Crantz) e reação de acessos de mandioca a *Xanthomonas axonopodis* pv. *manihotis* [Dissertação de Mestrado]. Brasília: Universidade de Brasília; 2014.

Bagamboula, C. F., Uyttendaele, M. & Debevere, J. Inhibitory effect of thyme and basil essential oils, carvacrol, thymol, estragol, linalool and p-cymene towards *Shigella sonnei* and *S. flexneri*. Food Microbiology. 2004; 21:33-42.
doi: https://doi.org/10.1016/S0740-0020(03)00046-7

Baldin, D. Extrato etanólico de própolis na proteção de feijoeiro ao crestamento bacteriano comum [Trabalho de Conclusão de Curso]. Laranjeiras do Sul: Universidade Federal Fronteira Sul; 2014.

Bianchini, L. & Bedendo, I. P. Efeito antibiótico da própolis sobre bactérias fitopatogênicas. Scientia Agricola. 1998: 55: 149-152.
doi: https://doi.org/10.1590/S0103-90161998000100024

Ceballos, L. F., Toro, J. C. & Silva, J. R. Sistema de propagação rápida de mandioca. Cali: CIAT – Centro Internacional de Agricultura Tropical; 1980. 12 p.

Celiktas, O. Y., Kocabas, E. E. H., Bedir, E., Sukan, F. V., Ozek, T. & Baser, K. H. C. Antimicrobial activity of methanol extracts and essential oils of *Rosmarinus officinalis*, depending on location and seasonal variations. Food Chemistry. 2007; 100: 553-559.
doi: https://doi.org/10.1016/j.foodchem.2005.10.011

Ferreira, D. F. Sisvar: a computer statistical analysis system. Ciência e Agrotecnologia. 2011; 35: 1039-1042.
doi: https://doi.org/10.1590/S1413-70542011000600001

Fonseca, M. G. Bioatividade de extratos de fungos da Antártica no combate a bacterioses da mandioca, tomate e pimentão causadas por *Xanthomonas* ssp. [Dissertação de Mestrado]. Rio Claro: Universidade Estadual Paulista Júlio de Mesquita Filho; 2018.

Gonçalves, D. S. Avaliação da atividade antibacteriana *in vitro* das própolis brasileiras verde, vermelha e marrom frente a *Xanthomonas citri* [Trabalho de Conclusão de Curso]. Uberlândia: Universidade Federal de Uberlândia; 2022.

Hillocks, R. J. & Wydra, K. Bacterial, fungal and nematode disease. In: Hillocks, R. J., Thresh, J. M. & Bellotti, A. C. (Eds.). Cassava: biology, production and utilization. New York: CABI International; 2002. p. 261-280.

Ishida, A. K. N., Cardoso, S. V. D., Almeida, C. A., Noronha, A. C. S. & Cunha, E. F. M. Incidência da bacteriose da mandioca (*Xanthomonas axonopodis* pv. *manihotis*) no estado do Pará. Embrapa Amazônia Oriental: Boletim de Pesquisa e Desenvolvimento; 2016. 22 p.

Kado, C. I. & Heskett, M. G. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas*. Phytopathology. 1970; 60: 969-979.

Koefender, J., Camera, J. N., Golle, D. P., Horn, R. C. & Dalazeri, P. Circular técnica: propagação rápida de mandioca tradicional do Alto Jacuí em diferentes substratos. Cruz Alta: Unicruz; 2017. 8 p.

Lima, R., Cardoso, M. G., Moraes, J. C., Vieira, S. S., Melo, B. A. & Filgueiras, C. C. Composição dos óleos essenciais de anis-estrelado *Illicium verum* L. e de capim-limão *Cymbopogon citratus* (DC.) Stapf: avaliação do efeito repelente sobre *Brevicoryne brassicae* (L.) (Hemiptera: Aphididae). BioAssay. 2008; 3: 1-6.
doi: https://doi.org/10.14295/BA.v3.0.56

Martini, D. Efeitos da sazonalidade nas características físico-químicas, potencial antibacteriano e antifúngico da própolis verde coletada em Campo Grande – MS [Dissertação de Mestrado]. Campo Bom: Universidade Anhanguera Uniderp; 2015.

Milos, M., Mastelic, J. & Jerkovic, I. Chemical composition and antioxidant effect of glycosidically bound volatile compounds from oregano (*Origanum vulgare* L. ssp. *hirtum*). Food Chemistry. 2000; 71: 79-83.
doi: https://doi.org/10.1016/S0308-8146(00)00144-8

Pereira, C. S. Bee products in plant propagation and control of gray leaf spot (*Cercospora coffeicola* Berk. & Cooke) and coffee leaf rust (*Hemileia vastatrix* Berk & Br.) [Dissertação de Mestrado]. Lavras: Universidade Federal de Lavras; 2004.

Portz, R. L., Kuhn, O. J., Franzener, G. & Stangarlin, J. R Caracterização de isolados de *Xanthomonas axonapodis* pv. *manihotis*. Acta Scientiarum. Agronomy. 2009; 28(3): 413-419.
doi: 10.4025/actasciagron.v28i3.965

Scazzocchio, F., D'auria, F. D., Alessandrini, D. & Pantanella, F. Multifactorial aspects of antimicrobial activity of propolis. Microbiology Research. 2005; 4: 327-333.
doi:10.1016/j.micres. 2005.12.003

Schauffler, G. P. Extratos de própolis no controle da mancha bacteriana (*Xanthomonas gardneri*) e da pinta bacteriana (*Pseudomonas syringae*) em tomateiro [Trabalho de Conclusão de Curso]. Florianópolis: Universidade Federal de Santa Catarina; 2017.

Silva, C. C. F. Caracterização química de quatro amostras de própolis brasileiras. Isolamento de substâncias e teste das atividades antioxidante e anti-HIV [Tese de Doutorado]. São Paulo: Universidade de São Paulo; 2013.

Silva, J. P. B. Avaliação do potencial antimicrobiano de extratos de própolis e do óleo essencial de *Melaleuca leucadendron* (L.) e proposição de um mecanismo de ação [Dissertação de Mestrado]. Ouro Preto: Universidade Federal de Ouro Preto; 2018.

Souza, L. S. & Fialho, J. F. Bacteriose podridão radicular e superalongamento. Embrapa Mandioca e Fruticultura. Sistemas de Produção. 2003; 8. Versão eletrônica. [Acesso em: 2 abr. 2023]. Disponível em: https://sistemasdeproducao.cnptia.embrapa.br/FontesHTML/Mandioca/mandioca_cerrados/doencas.htm#Superbrotamento.

Stangarlin, J. R., Schulz, D. G., Franzener, G., Assi, L., Schwanestrada, K. R. F. & Kuhn, O. J. Indução de fitoalexinas em soja e sorgo por preparações de *Saccharomyces boulardii*. Arquivos do Instituto Biológico. 2010; 77: 91-98.
doi: https://doi.org/10.1590/1808-1657v77p0912010

Tironi, L. F., Zanon, A. J., Alves, A. F., Freitas, C. P. O., Santos, A. T. L., Cardoso, O. S., Tonel, G. P., Rodrigues, L. B., Tagliapietra, B. L., Silva, M. N. & Streck, N. A. Ecofisiologia da mandioca visando altas produtividades. Santa Maria: Editora GR; 2019. 136 p.

Uzel, A., Sorkun, K., Önçag, Ö., Çogulo, D., Gençay, Ö. & Salih, B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiology Research. 2005; 60: 189-195.
doi: https://doi.org/10.1016/j.micres.2005.01.002