Chapter 7

Ralstonia solanacearum: A Bacterial Disease and Its Biological Control by Essential Oils on *Solanum tuberosum* L.

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Additional information is available at the end of the chapter

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Abstract

Worldwide, potato is considered the fourth most important crop for human consumption. In recent years, in some regions of the USA and Canada, the bacterium *Ralstonia solanacearum* (*Rs*), called bacterial wilt (Mb), has caused serious damage. Given the proximity of these countries, with Mexico as a tuber importer, the odds of an eventual introduction of these diseases are significant, especially in areas with large tracts of potato. Therefore, this research was performed to detect the presence of *Rs* in tuber and vegetative material of *Solanum tuberosum* and evaluated the bactericidal effect of essential oils. The results indicated that the presence of the bacterium *Rs* was negative in tuber from abroad. Nevertheless, we detected the presence of the causal agent of bacterial wilt in potatoes for domestic consumption that producers could use these tubers as production material. Oils of oregano and thyme showed inhibitory effects on the growth of *Rs*. Essential oils are considered as an alternative for the control of *Rs*.

Keywords: diagnosis, detection, phytopathogen, control, essential oils

1. Introduction

Global agriculture has been affected in recent years by phytosanitary problems caused mainly by fungi, bacteria, nematodes, weeds, and insects. With a radical change in international trade, and with the movement of vegetative material and sowing of these plant products, pests are dispersing throughout the world, becoming a more complex problem. Even when efforts are made, in the first instance to measure the introduction of diseases from other countries and secondly to

prevent their dispersion from primary inoculum sources, the negative aspects mentioned above have, to a certain degree, caused losses and major disruptions in some regions to national agriculture, so it is advisable to have the necessary measures to prevent the entry and secondly the control and dispersion of these biological agents harmful to plants [1]. In the Mexican Republic, specifically in northwestern Mexico, the area targeted for the cultivation of vegetables (mainly tomato, potato, chili, and watermelon) has increased considerably in recent years [2]. Mexico does not produce quality seed, it obliges producers to acquire tuber of foreign origin, mainly from the United States, since the varieties acquired produce fruits that meet the characteristics preferred by the consumer and also cause the tuber volumes to enter the country which is a gateway for microorganisms of quarantine importance such as *Pseudomonas solanacearum* = (currently) *Ralstonia solanacearum* to potato, *Xanthomonas campestris* pv. *vesicatoria* to chili and tomato, *Clavibacter michiganensis* ssp. *sepedonicus* to potato, *C. michiganensis* spp. *michiganensis* to tomato, and *Acidovorax avenae* pv. *citrulli* to watermelon, among others [1]. Also, the controversy is currently being generated among producers about their presence in agricultural fields in the state of Sonora, Mexico. Therefore, it is of great importance to know the current situation of *R. solanacearum* in potato (*Solanum tuberosum* L.), on material and during the different phenological stages of the crop in the agricultural zone of the state of Sonora, Mexico.

This research aims to expand knowledge of the situation that occurs in the detection of bacteria of quarantine importance, in addition to updating and reaffirming its null presence in the agricultural areas of the state, and later extend to the interior of the country. In addition, this screening study is aimed at involving producers with a new production scheme under phytosanitary conditions in order to safeguard our national agriculture.

On the other hand, in recent years, there has been a growing interest in the use of biologically active compounds extracted from plant species that have the ability to eliminate pathogenic microorganisms by themselves, mainly due to the resistance that microorganisms have developed to antibiotics [3]. In addition, agriculture in the new millennium must establish new control alternatives that produce a lower environmental impact, as day-to-day increases in the percentage of consumers who demand healthy and chemical-free food [4]. Therefore, the importance of knowing new control strategies arises, especially those that have a sustainable aspect. Based on the above, the need to evaluate bactericidal products to perform tests of antimicrobial activity against *R. solanacearum* is presented.

According to the abovementioned details, the principal goals were to detect the presence of *R. solanacearum* in tuber and vegetative material of *S. tuberosum* L. and to evaluate the bactericidal effect of essential oils.

2. Material and methods

2.1. Detection of brown rot (*Ralstonia solanacearum*) of potato crop (*Solanum tuberosum* L.) of the state of Sonora, Mexico

The research was carried out in two stages: the first one consisted in the increase of *R. solanacearum* (*Rs*) with specific culture media, tuber pathogenicity tests, seedling and fruit for familiarization

purposes in *Rs*, and their identification by ELISA technique. The second stage included a field sampled of tuber, seedling, flowering, and tubers produced in physiological maturity plants in Sonora state, to detect *Rs* by specific culture media and ELISA. Likewise, pathogenicity tests were carried out on those samples that were positive for the presence of *Rs*. Fit in mention that Detection of *Rs* in potato tuber was carried out in two types of tuber: (a) those from Canada and the United States of America (USA) and (b) tuber which it is to consumption human and used as a seed, it was sampled in commercial stores in Sonora state.

2.1.1. First stage

It was initiated with the increase of *Rs* and was developed according to the protocol established by Rueda [1], using means of specific cultures. According to the technique described by the same author, ten test tubes were obtained in 0.85% NaCl saline solution with a concentration of 108 colony-forming units (CFU)/ml, verified with the aid of a hematometer. Bacterial suspensions in tubes were stored in refrigeration at 4°C to stabilize the bacterium and avoid a shock in the immunization.

Pathogenicity tests (PPs) were developed in order to familiarize themselves with the symptomatology of *Rs* which were carried out in tuber and potato seedlings. In the case of tuber, 20 tubers were split in half and inoculated into the bundle by 200 ml with a bacterial suspension of 108 CFU/ml, and were placed in humid chambers under favorable conditions of the disease; likewise, another 20 tubers were considered as negative control when going through the same process but making use of sterile distilled water in the incision. Regarding PP in seedlings, 40 tubers were previously disinfected and were germinated in germination plates with sterile substrate, the conditions in which the seedling produced was at 25°C using sterile tap water for irrigation; at the end of 30 days after the emergency, 20 seedlings were inoculated with the bacterial solution of *Rs* at a concentration of 10^8 CFU/ml with the aid of a cotton swab on the cotyledons of the seedlings, and the remaining 20 seedlings were considered as negative control when passing through the same process with swab plus sterile distilled water. The tubers, seedlings used for PP, after inoculation, were covered with polyethylene bags and placed in an incubation chamber with a relative humidity between 80 and 90% and a temperature of 35–41°C in a period of 4–7 days [1]; these conditions are appropriate to induce the signs of the disease.

For the process of identification of *Rs*, the serological ELISA technique was developed, following the general protocol of identification of bacteria AGDIA. The *Rs* kit was donated by the project to which this research belongs.

2.1.2. Second stage

In the second stage, farmers donated tubers from Canada and the USA; the tuber, which is consumed by human and used as a seed, was sampled in commercial stores in Sonora state. Also, fields of potato crop were sampled in three stages: seedling, flowering stage, and physiological maturity when the plants produced tubers considered to be cut and later for sale. It should be noted that seedlings, leaves, or fruits of plants showing a symptomatology similar to that of *Rs* were also collected. Batch sampling was according to the National Potato Sampling in

Mexico [8]. Sampling was carried out in 10% of the total cultivated area of nine municipalities of Sonora state (Agua Prieta, Caborca, Cajeme, Hermosillo, Moctezuma, Navojoa, Sahuaripa, Santa Ana, and Ures). Located in the geographical coordinates, Agua Prieta 31° 17′ north latitude and 109° 33′ west longitude, Caborca 30° 42′ north latitude and 112° 09′ longitude west, Cajeme 27° 29′ north latitude and 109° 56′ longitude west, Hermosillo 29° 05′ north latitude and 110° 57′ longitude west, Moctezuma 29° 47′ north latitude and 109° 40′ west longitude, Navojoa 27° 03′ north latitude and 109° 25′ west longitude, Sahuaripa 29° 03′ north latitude and 109° 14′ west longitude, Santa Ana 30° 33′ north latitude and 111° 07′ west longitude, and Ures 29° 25′ north latitude and 110° 23′ west longitude. In the 10% of the surface of each municipality, the following was done: each batch of 5 ha was considered as a sampling area. Each hectare of that surface was a must-see. At each point an imaginary diagonal line was drawn from corner to corner, and on that straight line, ten samples were collected. Donations from producers were obtained. Each of the collected samples, previously identified, was wrapped with wet paper and placed in a cooler to be transferred to the laboratory for analysis.

Detection of Rs in tuber. According to Rueda [1], each tuber sample, consisting of 10 tubers from each batch of cooperating producers, weighed separately, washed in running water for 30 min, and placed in plastic trays with a capacity of 2 L. Each tray, with its respective sample of tuber, is left with an amount of 100 ml of distilled water, and each of these trays was added 2 ml of buffer solution phosphatase with a pH = 7. The water-phosphatase mixture containing each tuber sample is called a "mother suspension." The trays were incubated for 12 hours in cooling at 4°C in order to release the bacteria to the stock suspension. After the incubation, 10 ml was taken from suspension of each of the trays, four dilutions were made to such suspension (10:1, 10:2, 10:3, 10:4), and the last dilution of 0.1 ml was taken and sowed in specific culture medium in Petri dishes by the rod dispersion method The media were incubated for 7 days at 34°C. The inoculated media were then incubated at a temperature of 35°C for 7 days [1].

Detection of Rs in vegatitive material sampled (tuber, sedling, leaf and fruit), by ELISA technique. For the detection of *Rs*, with respect to the serological ELISA technique, the protocol described in the detection of the microorganism in the PP was considered.

Detection of *Rs* in tuber, seedling, leaf developed and fruit by PCR technique. Commercial primers were obtained from the 16Sr intergenic region, and screening tests were performed to reaffirm the null or positive presence.

Pathogenicity tests to positive bacteria with the different methods of detection. For the reaffirmation of *Rs* bacteria that proved to be positive in previous detection methods, they were carrying out pathogenicity tests [5]. The PPs were applied to seedlings 25–30 days after emergence, as described above in the PP of the first stage. The diseased tissue bacteria were reinoculated using the Randhawa technique, and the pathogen was confirmed by ELISA.

2.2. Evaluation of the in vitro antibacterial activity of essential oils of oregano and thyme against *Ralstonia solanacearum*

This stage which consisted of the evaluation of the antibacterial activity of two essential oils was carried out in the laboratory microbiology and mycotoxins of the Department of

Research and Postgraduate in Food of the University of Sonora, in the city of Hermosillo, Sonora, Mexico. The experiment was carried out in vitro under controlled conditions at 30 °C and 90% humedity.

The bacterial strain used in the study was *R. solanacearum*, was isolated, and characterized from pathogenicity tests of potatoes from commercial houses in the state of Sonora.

The bacterial strain was grown in a culture of 24 hours at 30°C in Nutrient Broth (Difco, Sparks, MD) (extract of 3.0 g and peptone of 5.0 g) and adjusted to a concentration of 10^8 CFU/ml with phosphate-buffered saline (PBS). The bacterial inoculum was massively planted on dextrose and potato agar plates using a sterile cotton swab to achieve uniform microbial growth [6].

Once the plates were inoculated with the bacteria, filter paper disks of approximately 10 mm in diameter were placed in the center of the dish, in which different amounts of the essential oils were applied.

Essential oils were prepared at different concentrations using 70% ethyl alcohol as diluent. The concentrations used were 1:1, 1:5, and 1:10. Aseptically, 7.5, 10, and 15 μl of each of the concentrations of the essential oils were placed on the filter paper disks. Seventy percent alcohol was used in one of the filter paper disks as a negative control to discard the antimicrobial activity of the same. In addition, a disk of streptomycin (10 μg/disk) and one ampicillin (10 μg/disk) were used as the reference control. After impregnating the disks with the respective treatment, the plates were incubated at 30°C for 24 hours. After the incubation period, bacterial growth inhibition halos were measured in millimeters using a ruler. Analyses were carried out in triplicate.

The experimental design was trifactorial A × B × C where factor A has two oils [oregano (*Lippia graveolens*) and thyme (*Thymus vulgaris*)], factor B has three dilutions (1:1, 1:5, and 1:10), and factor C has three amounts applied 7.5, 10, and 15 μl. The data were analyzed in Statistix 8.0 program (2003).

3. Results

3.1. Detection of brown rot (*Ralstonia solanacearum*) of potato crop (*Solanum tuberosum* L.) of the state of Sonora (Mexico)

3.1.1. First stage

When testing for pathogenicity for *R. solanacearum* (*Rs*) symptoms in potato seedlings, the results indicate that between the tubers embedded in the bacterial suspension of 108 CFU/ml and between 7 and 15 days under favorable conditions of the disease, the vascular bundle of the tuber was darkened, and, when making a cross section, a grayish bacterial mucilage was exuded by the eyes and by the end of the stolon in the tubers. There were grayish-white outcrops that exudate from the darkened vascular ring of the cut tubers. On the other hand, in the inoculated seedlings when making a transverse cut at the stem level, the exudation of a gray-brown mucilage was noted. This could be verified by makng a transverse cut at the base of the

stem of the seedling and observing a milky-white filamentous fluid emanating from the vascular bundles and submerging a piece of the stem in clean, sterile water. In the same way, in the first true leaves for the seedling case, irregular spots of whitish appearance were observed in relation to the healthy area, and finally the death of the seedling occurred. For this, first appeared irregular spots at first clear and then obscure, and after 7–14 days death occurred. An opposite result was for those PPs that were directed as negative control by the use of sterile distilled water, in tuber-tubercle and seedling, being observed that the organs used were shown healthy, except in tuber where the punctured area showed a light brown oxidation (2 mm) which, when pressed, showed the same solid consistency as the healthy area [7].

Regarding the sowing of "bacterial suspensions" obtained from the pathogenicity test on media Mannitol salt agar (SMSA), and tetrazolium chloride (TZC) cultures, the results indicate that on the TZC medium, developed colonies showed a pinkish-white color, while on the SMSA medium, colonies of developed bacteria showed an irregular shape, of white color and with centers of pink color after 72 hours of incubation at 30°C. A similar result was obtained for those PPs that were inoculated with the positive control (*Rs*), whereas for those samples generated from the PPs that served as negative control (sterile distilled water), the results on both media used were non-mucoid dry colonies [7].

By ELISA confirmation, a positive result was obtained for the control strain (*Rs*), as well as for the bacterial suspensions isolated from the aforementioned pathogenicity tests and from those grown on the culture media SMSA and TZC. The opposite happened with the negative control [7].

3.1.2. Second stage

Sampling of potatoes was carried out in the municipalities of Agua Prieta, Caborca, Cajeme, Hermosillo, Moctezuma, Navojoa, Sahuaripa, Santa Ana, and Ures according to the sampling method [8] and detection of *Rs* (**Table 1**). It indicates the surface that is directed to the production of potato in the state of Sonora. The sampled area (889.8) can be identified, with the municipality of Navojoa and Cajeme with 394 and 240.6 ha, respectively [7].

The results of the phytopathological diagnosis show positive *Rs* for the municipalities of Navojoa, Hermosillo, and Agua Prieta in a consumption tuber that can be used as seed (**Table 2**). When the tests were carried out on the SMSA, TZC, and PPO media, the results were positive since the visible colonies on SMSA medium were after 36–48 hours of growth at 30°C, white with pink to colored centers of cream and irregularly round; on the TZC medium, the colonies appeared white with pink centers. It is possible to indicate that those bacterial colonies grown on SMSA and TZC medium obtained from samples of potatoes for consumption indicated that the bacterial cells were Gram-negative, in a bar form, strictly aerobic, and with measurement of 0.5–0.7 × 1.5–2.0 μm. These colonies were tested positive by ELISA and PCR [7].

In import tuber, the results were negative. Nonetheless, it is important to note that in response from abroad, there was variability of response for the municipalities of Agua Prieta, Sahuaripa, Moctezuma, Hermosillo, and Ures, as they were positive with the specific medium TZC but negative to the PPO and ELISA test [7].

Regarding the analyses carried out in seedlings, a leaf developed during the flowering stage and tubercle of fruit sampled at physiological maturity, the results indicate that for samples

District	Area sowed (ha)				
	Total	Irrigation	Raining area	Surface sampled from irrigation area 10%	Surface sampled from raining area
Navojoa	3940	3940	0	394	0
Cajeme	2406	2406	0	240.6	0
Hermosillo	371	371	0	37.1	0
Ures	115	115	0	11.5	0
Caborca	2000	2000	0	200	0
Agua Prieta	35	35	0	3.5	0
Sahuaripa	5	0	5	0	0.5
Santa Ana	30	30	0	3	0
Moctezuma	1	1	0	0.1	0
Total	8903	8898	5	889.8	0.5

Table 1. Surface sowed by potato crop in different districts of Sonora state (surface sampled to detect *Ralstonia solanacearum*).

obtained from the municipalities of Agua Prieta, Navojoa, Hermosillo, and Caborca, they were negative when using specific media (SMSA and TZC) and serological tests, except in the PPO test that was positive for leaf and fruit (**Table 3**) [7].

On the other hand, when analyzing the vegetative samples from Sahuaripa, Cajeme, and Ures, the analyses showed negative results in specific medium SMSA and serological tests, the opposite occurred in culture medium TZC and PPO test. An additional test was developed to the isolated colonies of the different sampling points, resulting being Gram-negative, in a bar form, strictly aerobic, and with measurement of 0.3–0.5 × 1.0–1.5 μm. For the municipality of Moctezuma, the results indicate the negative presence of *Rs*. A favorable result was obtained for the positive control in the tests SMSA, TZC, PPO, and ELISA [7]. Concerning the PP of the positive samples of consuming tuber, tubers embedded in the bacterial suspension of 10^8 CFU/ml, between 5 and 15 days under favorable conditions of the disease, presented a wateriness in the vascular bundle of the tuber. It was also detected that in tubers inoculated with *Rs*, whitish exudates of paste consistency appeared. As the infection evolved, there was a darkening of the entire vascular ring, and the adjacent tissues began to decompose, presenting a yellowish, creamy, or brownish coloration, eventually ending up rotting. When corroborating these symptoms by the ELISA technique, the result was positive for *Rs*. In the case of seedlings, the pathogenicity tests showed, in the first true leaves, irregular spots of whitish appearance relative to the healthy area and finally the death of the seedling. For this, irregular, initially clear and subsequently obscure patches were first presented, slight yellowing of the bacterial wilt disease of the plant, which is observed first on a single side of the leaf or on a branch, and after 7–14 days, death occurred. A positive result for *Rs* was obtained by ELISA, when analyzing the organs of PP in the seedling stage. The opposite occurred for those PP organs inoculated with sterile distilled water [7].

	Technical of diagnosis			
	Test SMSA	**Test TZC**	**Test PPO**	**Serology technique**
Potato from Canada and the USA				
Navojoa	−	−	−	−
Cajeme	−	−	−	−
Hermosillo	−	+	−	−
Ures	−	+	−	−
Ures	−	+	−	−
Caborca	−	−	−	−
Agua Prieta	−	+	−	−
Sahuaripa	−	+	−	−
Santa Ana	−	−	−	−
Moctezuma	−	+	−	−
Potato tuber's sampled in commercial stores in Sonora state, which is for human consumption and used as a seed				
Navojoa	+	+	+	+
Cajeme	−	−	−	−
Hermosillo	+	+	+	+
Ures	−	−	−	−
Caborca	−	−	−	−
Agua Prieta	+	+	+	+
Sahuaripa	−	−	−	−
Santa Ana	−	−	−	−
Moctezuma	−	−	−	−
Control positivo *Rs*	+	+	+	+
Control negativo (distilled water)	−	−	−	−

+, Test positive; −, test negative; specific media, SMSA and TZC. *Rs*, *Ralstonia solanacearum*; PPO, oxidase test.

Table 2. Detection of *Ralstonia solanacearum* in potato tuber from Canada and the United States of America (USA) and tuber of potato sampled in commercial stores in Sonora state, which is for human consumption and used as a seed.

3.2. Evaluation of the in vitro antibacterial activity of essential oils of oregano and thyme against *Ralstonia solanacearum*

Both oregano and thyme essential oils presented inhibition halos, such as antimicrobial activity against *R. solanacearum*, at all concentrations and amounts applied.

Districts sampled	Techniques of diagnosis			
	Test SMSA	Test TZC	Test PPO	Serology technique
Navojoa				
Seedling	–	–	–	–
Leaf	–	–	+	–
Fruit	–	–	+	–
Cajeme	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Hermosillo	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Ures	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Caborca	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Agua Prieta	–	–	–	–
Seedling	–	–	+	–
Leaf	–	–	+	–
Fruit				
Sahuaripa	–	+	+	–
Seedling	–	+	+	–
Leaf	–	+	+	–
Fruit				
Santa Ana	–	–	–	–
Seedling	–	–	–	–
Leaf	–	–	–	–
Fruit				
Moctezuma				
Seedling	–	–	–	–
Leaf	–	–	–	–
Fruit	–	–	–	–
Control positive *Rs*	+	+	+	+
Control negative *Rs*	–	–	–	–

+, Test positive; –, test negative; specific medium, SMSA and TZC. PPO, oxidase test; control negative = sterile distilled water.

Table 3. Detection of *Ralstonia solanacearum* in seedling, leaf, and fruit sampled from crop potato areas in Sonora (Mexico).

The average antibacterial activity obtained from all concentrations and different amounts of oregano extract applied was 25.8 mm. The mean inhibition values found were 38.3, 20.5, and 20.2 mm in diameter, for concentrations 1:1, 1:5, and 1:10, respectively. In a similar study in which the antibacterial activity of the oils of four different varieties was evaluated, oregano on *C. michiganensis* subsp. *michiganensis* (Cmm) bacteria average values of 47.5, 35.6, and 30.8 mm in diameter for the same concentrations and in the amount used applied 15 μl [6] were found. These inhibition results are greater than those found in the present experiment; this may be due not only to the different sensitivity of the bacteria under study but also to the different composition of the oregano oils used. The concentration of essential oil of oregano showed that the greater antibacterial activity against *R. solanacearum* was 1:1 in different amounts applied with 7.5 μl with 35.16, 10 μl with 39.15 mm, and 15 μl with 40.83 mm in diameter. On the other hand, the results of inhibition of *R. solanacearum* were very similar when they were applied with 7.5 and 10 μl of the extract in concentrations 1:5 (average value, 19 mm) and 1:5 (average value, 19.5 mm). However, the amount applied had the greatest effect when 15 μl of the extract was applied, especially in the 1:5 concentration (23.8 mm).

The average antibacterial activity obtained from all concentrations and different amounts of thyme extract evaluated was 26.2 mm. The mean inhibition values found were 36.3, 28.0, and 14.1 mm in diameter, for the concentrations 1.1, 1:5, and 1.10, respectively. Our results were lower compared with [6], which obtained 50.3, 33.0, and 21.0 mm for the same concentrations to *C. michiganensis* subsp. *michiganensis*.

The concentration of thyme oil that showed the greatest halo of growth inhibition of the bacterium *R. solanacearum* was the 1:1 concentration in the different amounts applied, with 33.5, 35.2, and 40.5 mm, when 7.5, 10, and 15 μl, respectively, as shown in **Figure 1(a)**.

When comparing the essential oils of oregano (*L. graveolens*) and thyme (*T. vulgaris* L.) with respect to mean halo values of inhibition in the growth of *R. solanacearum* ($P > 0.05$), there is no significant difference in oregano (25.907) and thyme (27,204). The two oils are considered to have an inhibitory effect on the growth of *R. solanacearum*. Other studies also showed inhibitory capacity in bacterias such as *Agrobacterium tumefaciens*, *C. michiganensis* subsp. *michiganensis*, *Erwinia amylovora*, *E. carotovorum*, *E. Xanthomonas vesicatoria* [9–11]. Regarding the comparison of the average values of the dilutions (1:1, 1:5, 1:10) of the essential oils of oregano and thyme in halo of inhibition of growth of *R. solanacearum* (**Figure 1(a, b)**), the resoults showed a significant difference (p0.05) in the 1:1 dilution. In addition a similar result with the 1:1 dilution was obtained in the growth inhibition study of *C. michiganensis* subsp. *michiganensis* with oregano and thyme oils [6].

In the comparison of the mean values of applied amounts (7.5, 10.15 μl) of oregano and thyme essential oils in growth inhibition halo of *R. solanacearum* ($P < 0.05$), there was a significant difference in applied amount of 15 μl:30, 7.5 μl:22.7, and 10 μl:26.05 (**Figure 1(a, b)**). The growth inhibition halo values of the bacterium *R. solanacearum* by the effect of oregano and thyme oils

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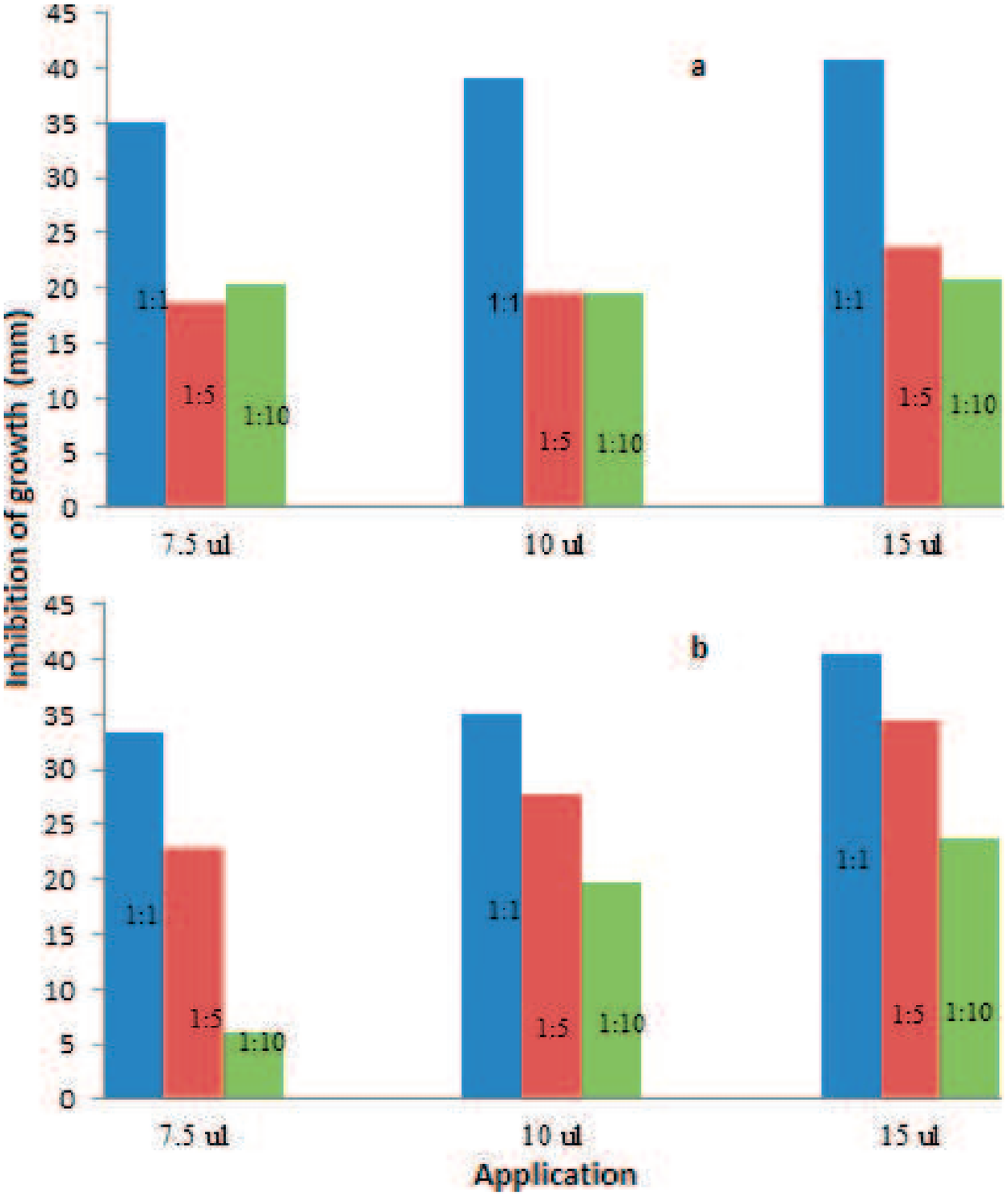

Figure 1. Inhibition of growth of *Ralstonia solanacearum* by oils of oregano (a) and thyme (b) in dilutions of 1:1, 1:5, and 1:10 at 7.5, 10, and 15 μl applied at 24 hours.

are shown in **Table 4** in which it is indicated that ($P > 0.05$) there is no significant difference between oregano stockings 40,833 and thyme 40,500 ha.

Essential oil	Dilutions	Applications	Values
Oregano	1:1	15 μl	40.833 a
Thyme	1:1	15 μl	40.500 a
Oregano	1:1	10 μl	39.000 ab
Thyme	1:1	10 μl	35.167 abc
Oregano	1:1	7.5 μl	35.167 abc
Thyme	1:5	15 μl	34.500 abcd
Thyme	1:1	7.5 μl	33.500 abcde
Thyme	1:5	10 μl	27.833 abcdef
Thyme	1:10	15 μl	24.500 bcdef
Oregano	1:5	15 μl	23.833 cdef
Thyme	1:5	7.5 μl	23.000 cdef
Oregano	1:10	15 μl	20.833 def
Oregano	1:10	7.5 μl	20.333 ef
Thyme	1:10	10 μl	19.833 efg
Oregano	1:5	10 μl	19.500 efg
Oregano	1:5	7.5 μl	18.667 fg
Oregano	1:10	10 μl	15.000 fg
Thyme	1:10	7.5 μl	6.000 g

Values with the same letter does not exist significance (P > 0.05).

Table 4. Inhibition of growth of *Ralstonia solanacearum* by essential oils (trifactorial A × B × C).

4. Conclusion

The presence of *Rs* bacterium was proven to be negative in tuber from abroad. Nevertheless, the presence of the causative agent of bacterial wilt in potatoes of national consumption was detected that some producers could use tuber. The presence of *R. solanacearum* was verified through the use of specific culture media called SMSA and TZC under controlled conditions, PPO test, and pathogenicity tests. It is concluded that separate screening tests should not be used as a single detection method. *R. solanacearum* was found to be positive in the tubercle of consumption, in the different vegetative stages (seedling, leaf developed, and fruit tuber) in which samples were taken for detection of bacterial disease; these were negative in all cases, being corroborated under the same detection techniques implemented. However, since the presence of *Rs* in consumption tubers is positive, it represents a risk of a possible manifestation of the disease; it is necessary that the producing areas carry out activities to prevent the disease from developing, such as certified tuber verification, cleaning, and disinfection of machinery, among others, and, even more, to test on imported potatoes to prevent the entry of tuber-containing bacteria.

According to the essential oils of oregano and thyme, they showed inhibitory effects on the growth of the bacterium *R. solanacearum* at the 1:1 dilution result ($P < 0.05$) to be more effective than the rest of the dilutions evaluated, and the most effective applied amount was 15 μl of oregano and thyme essential oil.

The essential oils of oregano and thyme showed ($P < 0.05$) better inhibitory effect than the antibiotics used streptomycin (10 μg) and ampicillin. Therefore, essential oils are excellent alternatives to antibiotics in the control of the bacterium *R. solanacearum*. However, it is very important to consider others studies to evaluate the phytotoxic activity of essential oils studied in this research (*L. graveolens* and *T. vulgaris*) on *Rs* in different phenological stages of potato crop under production system.

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