



Management of *Erwinia amylovora* by Potential Bio-Pesticides *in vitro* and *in vivo* Conditions[#]

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ABSTRACT

Erwinia amylovora, the causative agent of fire blight disease, threatens a lot of species of the *Rosaceae* family. Antibiotics and copper compounds in chemical applications are most frequently applied, but these can be phytotoxic and cause resistant strains of the pathogen. In our experiments, 20 herbal materials were tested for their antimicrobial effectiveness against the fire blight pathogen *in vitro* and *in planta*. The air-dried plants ground into fine powder and extraction was performed at room temperature by maceration with 80% (v/v) methanol/distilled water. The minimum inhibitory concentration values were determined by using disc diffusion method and streptomycin was used as control in all experiments. Antimicrobial activity was evaluated by measuring the inhibition zones in reference to the pathogen. Among the tested plants, *Szygium aromaticum*, *Thymus vulgaris* and *Rhus cararia* showed a good antibacterial activity and they inhibited the growth of *E. amylovora* with inhibition zone diameter ranging from 21 to 27 mm at 20% (w/v) in absolute methanol compared to streptomycin (31 mm) *in vitro* conditions. *In vivo* tests were performed by using highly virulent *E. amylovora* isolate (Eak24b, 91%) grown on TSA medium and inoculation on young shoots of 3-year-old Gala variety of apple and Santa Maria variety of pear seedlings at 10⁷ CFU ml⁻¹ density of the pathogen. Disease severity (%) was assessed by proportion of blighted shoot length to the whole shoot length and also efficacy of the extracts was determined by using Abbott formula. The highest efficacy was obtained by *S. aromaticum* and *T. vulgaris* extracts of reducing shoot blight of cv. Gala and cv. Santa Maria by 67.81% - 64-12% and 51.50% - 51.04% ratios, respectively. Obtaining results showed that some medicinal and aromatic plant extracts might be used against fire blight disease as potential new generation chemicals on pome fruits within integrated and organic control programs.

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Introduction

Apple is one of the most widely grown fruit tree species in the genus *Malus* of the family *Rosaceae* with more than 7,500 known cultivars of apple. Apple originated in Central Asia and widely consumed, is one of the most important fruit species and its production takes a considerable part in agriculture production in the world. China is the most important producer country with about 39.2 million tones of apples and followed by USA (4.7), Poland (4.0), Turkey (3.6), Iran (2.5), Italy (2.4) and India (2.3), respectively (Anonymous, 2020; FAO, 2018). In addition, South and Central Anatolia, Black Sea Coast Region and transition regions between East Anatolia uplands constitute the most important apple growth area in Turkey (Oguz et al., 2009).

Pears are the second most important fruit species in *Rosaceae*, with world production of approximately 17.2 million tons in area of 1.381.923 ha annually (FAO, 2018)

and pear is the ninth most important cultivated fruit in the world. There are twenty-two species of the genus *Pyrus* and originated in Asia, Africa and Europe. Four important *Pyrus* species are commercially grown for edible fruit; Japanese pear (*P. pyrifolia* Nakai), European pear (*P. communis* L.), and Chinese pears (*P. bretschneideri* Rehd. and *P. ussuriensis* Maxim.) (Bell et al., 1996). China is the world's largest producer and it is followed by the United States as being the first producer of European pear type. Bartlett pear is the most common throughout the world accounting for about 75% of all pear production (Silva et al., 2014).

Apples and pears have a number of diseases and they appear depending up on the weather conditions and the development or phenology of the apple, beginning in early stages and continuing until harvesting. The diseases, are

common in apple include fire blight and apple scab. Of these diseases are generally more difficult to control in years of prevailing high temperature, high humidity, and abundant rainfall and cloud-cover. A season long program for disease management is necessary in order to healthy fruit production (Douglas, 2018).

Fire blight caused by *Erwinia amylovora* (Burr.) Winslow et al., is the most serious bacterial disease of apple, pear, quince, sorbus, hawthorn, pyracantha, cotoneaster, creteagus and other about 130 plant species in the family *Rosaceae* (van der Zwet and Keil, 1979; Vanneste, 2000). The disease, which has a history of approximately 250 years, was first detected in the pear orchards in Sultandağı district of Afyon province, Turkey in 1985 (Oktem and Benlioglu, 1988).

The pathogen is included among quarantine organisms in many countries and very strict quarantine measures are forced (Smith et al., 1991). Since sanitation methods could not stop the spreading of the disease, fire blight management using appropriate chemicals and bio-control agents is the focus of ongoing efforts. The most effective control, but for a short time, can be achieved through streptomycin applications (Johnson and Stockwell, 1998). However, streptomycin use has been prohibited in many countries due to the risk of resistance development in the population of *E. amylovora* and non target beneficial bacteria (Iacobellis et al., 2005). Copper compounds, which are applied frequently, are not sufficiently effective in the disease management for apples and could have unfavourable effects either on the environment, on human and animal health and on phytotoxic effects on the plants (Loper et al., 1991; Saygılı and Ustun, 1996; Iacobellis et al., 2005; Vanneste et al., 2005). Because of these aspects, it is need for alternative control strategies including natural compounds (Zeller and Laux, 2001).

Herbal essential oils and extracts, which have strong antibacterial activity and are environmentally friendly, have provided promising results in the combat against plant pests as well as plant and food-borne disease agents (Soylu et al., 2010). Today, there are many biopesticides that have been developed and released to the market. In addition to their rapid and clear effects, their affordable prices have made them popular (Isman, 2006).

In recent years, searches of eco-friendly biological alternative on control of the fire blight disease are oriented to the use of promising herb extracts/essential oils have a strong antimicrobial activity (Scorhictini and Rossi, 1989; Mosch et al., 1989, 1996; Kokoskova et al., 2011; Rafaat et al., 2015). Plant extracts for the management of plant diseases are environmentally safe, long lasting and extracts of certain plants contain alkaloids, phenolic compounds and phytoalexins.

The presence of antibacterial compounds in higher plants has long been recognized as an important factor in disease control and their toxicity has not determined till now (Dorman and Deans, 2000; Gwinn, 2018).

In this study, it was aimed to the determine of antibacterial efficacy of successful extracts from some medicinal and aromatic herbs *in vitro* conditions and on susceptible apple cultivar Gala and pear cultivar Santa Maria compared to streptomycin against *E. amylovora*.

Material and Methods

Plant Material

Medicinal and aromatic plants were collected between May and August 2017 from culture gardens of Konya province and the research field of Selcuk University and, botanists and herbologists from Selcuk Univ identified the herbs. After pre-experiments *in vitro*, the most effective 20 plants; the leaves of ivy (*Hedera helix* L.), lavender (*Lavandula officinalis* L.), eucalyptus (*Eucalyptus globulus* Labill.), Izmir oregano (*Origanum onites* L.), daisy (*Matricaria recutita* L.), mint (*Mentha arvensis* L.), lemon balm (*Melissa officinalis* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), summer savory (*Satureja hortensis* L.) and thyme (*Thymus vulgaris* L.), seeds of mustard (*Brassica nigra* L.) nigella (*Nigella sativa* L.) and cumin (*Cuminum cyminum* L.), fruits of onion (*Allium cepa* L.), garlic (*Allium sativum* L.), sumac (*Rhus coriaria* L.) and basil (*Ocimum basilicum* L.), flower buds of clove (*Syzygium aromaticum* L.) and bark of cinnamon (*Cinnamon zeylanicum* L.) were used *in vitro* and *in vivo* tests of this study.

The leaves, seeds, root and fruits of medicinal and aromatic plants, which are subjected to the experiments, were dried in the laboratory in a shady and airy environment for 6-7 days. These materials were then milled and put into glass jars as 50 g. The jars, which were covered with aluminum foil by adding 500 ml of methanol, were kept in a dark environment and at room temperature by shaking for 5-6 days. At the end of this period, the suspensions obtained were filtered through filter paper and liquid parts were taken. The methanol in the filtrate was evaporated with the help of the Rotari evaporator at 40±2 temperature and the plant extracts were obtained (Tavares et al., 2009). Then, they were diluted with dimethyl sulfoxide (DMSO, Merck) and prepared in five concentrations of 20%, 10%, 5%, 2.5% and 1% (w/v) and they were used in the experiments. The plant extracts obtained were stored at 4 °C and darkness until using.

Three-year-old apple cv. Gala and pear cv. Santa Maria seedlings, which are shown uniform growth, were used *in vivo* tests. The seedlings were grown in a greenhouse in 45-cm-diameter pots containing a sterilized mix of soil-sand-peat (2:1:1 by volume) and watered daily by drip-irrigation. A mineral solution (NPK 20-20-20) at 2 g l⁻¹ was distributed weekly into the pots to maintain optimum nutritional conditions.

E. amylovora Isolate and Growth Conditions

Highly virulent *E. amylovora* isolate, Eakb24 (91%) used in the assays was obtained from phyto bacteriology culture collection of the Department of Plant Protection, Selcuk University. The pathogen was grown on nutrient broth (NB) at 25±2°C. It was used bacterial suspensions prepared from 48-hour fresh of Eakb24 isolate adjusted to 10⁷ CFU ml⁻¹ concentrations in a spectrophotometer (Eppendorph Bioplus, OD600: 0.2).

In vitro Antibacterial Assays

The antibacterial effect of plant extracts on *E. amylovora* was investigated using *in vitro* petri dishes using the paper diffusion disc method (Bauer et al., 1966; Mangamma and Sreeramulu, 1991). 100 µl of *E.*

amylovora suspension containing 10^7 CFU ml^{-1} populations was spread to 9.0 cm diameter petri dishes containing King's B medium by drigalski spatula. After the petri dishes were dried, a round sterile paper disk with a diameter of 1 cm was placed at three separate points on the petri dishes at equal distance from each other. 10 μl of herbal extract were taken and dropped on a paper disc. Sterile water was used as negative control and streptomycin (0.02 g L^{-1}) as positive control. The experiment was set up using three petri dishes, three paper discs in each petri dish. After the petri dishes were incubated at $25 \pm 2^\circ\text{C}$ for 48 hours, the inhibition zones formed around the paper discs were measured and noted in mm. The lowest concentration of the extract (%) that prevented the visible growth of *E. amylovora* was considered as the minimum inhibitory concentrate (MIC). After the inhibition zones formed by the plant extracts were measured in mm, the antibacterial effect index (AEI) was compared with the positive control and calculated according to the formula below (Kokoskova and Pavela, 2007). The AEI rate is referred to the rate of effect of the applications relative to streptomycin. The antimicrobial efficacy index (AEI) was calculated with the formula:

$$\text{AEI (\%)} = \{-1 \times [(C-T)/(C+T)]\} \times 100$$

where C is the average inhibitory zone (cm) on the positive control dish (streptomycin) and T is the average inhibitory zone on the treated dish.

Inoculation of the Shoots and Application of the Plant Extracts In vivo

According to *in vitro* assays, the most successful selected three herbal extracts (*Szygium aromaticum*, *Thymus vulgaris*, *Rhus coriaria*) were used at a concentration of 20% concentration. The apple seedlings were sprayed with plant extracts once time 5 days ago from the inoculation of *E. amylovora* and after the inoculation two times with 5 days intervals, totally 3 times. The plants were kept for five days at 85% RH, 25°C and 16 h of daylight. Control plants were sprayed by sterile distilled water.

Inoculum was prepared from 48-h-old bacterial cultures grown on NB medium at $25 \pm 2^\circ\text{C}$. The inoculum suspension was homogenized and suspended in sterile distilled water to adjust 10^7 CFU ml^{-1} as described above.

Shoots were inoculated by transversally bisecting the two youngest actively growing leaves with scissors and dipped 30 sec in suspension of Eakb24. The treated shoots were labeled with flagging tape for evaluation purposes (Zeller and Meyer, 1975; Norelli et al., 1984).

Re-isolation of the Pathogen

Re-isolations were made from sections of symptomatic leaves, shoots, and control plants after surface-sterilization the tissue sections by dipping 70% ethanol for 1 s, followed by rinsing three times in sterile distilled water. Then, a 1 g subsample of each tissue section was homogenized in 10 ml phosphate buffered saline for 30 min. From each homogenate, an aliquot of each dilution was plated onto 5% Nutrient Sucrose Agar (NSA) medium, and the plates were incubated for 2 days at 25°C . Re-isolated bacteria were identified on the basis of biochemical and molecular tests. A

reference strain of *E. amylovora* (NCPPB 2791) was used as positive control in the tests. The specific oligonucleotide primers, A: 5'CGGTTTTTAACGCTGGG3' and B: 5'GGGCAAATACTCGGATT3' were used in PCR assays (Bereswill et al., 1995; Schaad et al., 2001).

Experimental Design and Setup

The experiment was set up in a completely randomized block design with three replicates. A single replicate was a mean from nine shoots on three saplings.

Evaluation of Disease Severity and Shoot Growth

The length of visible fire blight lesions and of the current season's shoot growth was recorded after all lesions had ceased to extend, as indicated by the formation of a determinate margin between diseased and healthy tissues. Percent disease severity (DS) was calculated using the following formula:

$$\text{DS (\%)} = (a / b) \times 100$$

where a is the length of the blighted part of the shoot (cm), and b is the whole length of the shoot (cm) (Fernando and Jones, 1999; Aldwinckle et al., 2002).

Percent effectiveness of the applications (A) was calculated according to the following formula (Abbott, 1925):

$$\text{A (\%)} = (B - C) / B \times 100$$

where B is the percent disease severity in the controls, and C is the percent disease severity in treated shoots

Percent effectiveness of the treatments on shoot growth (D) was calculated similarly:

$$\text{D (\%)} = (E - F) / E \times 100$$

where E is the mean shoot length in the controls, and F is the length of treated shoots

Statistical Analysis

Variance analysis of the data obtained from the study using MINITAB ver. 18 program and statistical evaluations with Tukey multiple comparison test in the MSTAT program were used to analyse between interactions the chemicals and the disease severity determined (Düzgüneş ve ark., 1987). Significant differences between the two mean values, due to different treatments or varieties and their interaction at a crop growth stage, were computed by comparing their significant levels at $P \leq 0.05$.

Results

In vitro Assays

Antibacterial efficacy of some medicinal and aromatic plant extracts on *E. amylovora* was evaluated using the disc diffusion method to measure the surrounding inhibition zones at 5 doses *in vitro* conditions (Table 1). The mean values of the inhibition zones caused by extract to the bacterial agent ranged from 0 to 25 mm. The inhibition zone increased in a dose-dependent manner for all extracts. Three of the twelve plant extracts were taken to the

experiment were found to have antibacterial activity by forming an inhibition zone between 5 and 25 mm against *E. amylovora*. The maximum inhibition zones for the inhibition of the pathogen growth was obtained with the 20% concentration of *S. aromaticum* (27 mm) and *T. vulgaris* (24 mm) extracts and they showed the closest effect to streptomycin (31 mm) according to IAE (%) (Table 1 and Figure 1). The lowest inhibition zones of 6, 8 and 9 mm were obtained from *Lavandula officinalis*, *Nigella sativa* and *Origanum onites* at the same concentration dose of extracts, respectively.

In *in vitro* studies, the plant extract, which was prepared from *S. aromaticum*, showed the largest inhibition zone diameter (27±1.19 mm) and strongest antibacterial effect among the tested plant extracts, which is almost near to the inhibition effect of the positive control streptomycin (31±1.7 mm). It was followed by *T. vulgaris* (24 ±1.46 mm) and *R. cararia* (21±1.54 mm), respectively. These plant extracts, which are effective in preventing the development of the pathogen under *in vitro* conditions, have been evaluated as promising in the control against the disease.

Table 1. Zones of growth inhibition (mm) showing antibacterial activity of plant extracts against *Erwinia amylovora* on paper disc diameter of 5.0 mm

Plants	Concentrations of the plant extracts (%)					MIC	IAE (%)
	20	10	5	2.5	1		
<i>Lavandula officinalis</i>	6±1.12 ^{g*}	1±0.18	0.0	0.0	0.0	10	-67
<i>Nigella sativa</i>	8±1.91 ^{efg}	1±0.84	0.3±0.79	0.0	0.0	5	-58
<i>Origanum onites</i>	9±2.87 ^{ef}	2±0.13	0.6±0.90	0.0	0.0	5	-55
<i>Cinnamon zeylanicum</i>	9±1.73 ^{ef}	5±1.78	1±1.06	0.0	0.0	5	-55
<i>Ocimum basilicum</i>	10±1.06 ^e	4±0.13	1.5±0.92	0.0	0.0	5	-51
<i>Matricaria recutita</i>	10±3.12 ^e	3±3.14	2±0.89	0.0	0.0	5	-51
<i>Hedera helix</i>	10±2.69 ^e	4±1.45	0.5±1.2	0.0	0.0	5	-51
<i>Brassica nigra</i>	11±1.0 ^{de}	5±1.6	2.5±1.72	1±0.88	0.0	2,5	-47
<i>Cuminum cyminum</i>	12±2.34 ^{de}	5±1.18	1±1.19	0.0	0.0	5	-44
<i>Satureja hortensis</i>	13±1.59 ^d	7±0.96	4±0.89	1±0.91	0.0	2,5	-40
<i>Salvia officinalis</i>	13±1.73 ^d	5±1.78	1±1.06	0.0	0.0	5	-40
<i>Eucalyptus globulus</i>	16±2.01 ^{cd}	11±2.17	7±1.17	1±0.72	0.0	2,5	-31
<i>Melissa officinalis</i>	17±1.45 ^c	11±0.46	8±1.45	1±2.01	0.0	2,5	-29
<i>Mentha arvensis</i>	18±1.12 ^c	12±0.93	9±2.24	1.5±1.3	0.0	2,5	-26
<i>Rosmarinus officinalis</i>	18±1.02 ^c	11±1.21	7±0.59	2±0.67	0.0	2,5	-26
<i>Allium cepa</i>	19±2.3 ^{bc}	12±0.79	8±2.06	2±1.41	0.0	2,5	-24
<i>Allium sativum</i>	20±2.16 ^{bc}	12±1.14	8±1.9	2.5±0.1	0.5±0.8	1	-21
<i>Rhus cararia</i>	21±1.54 ^{bc}	14±1.97	6±0.61	1±1.25	0.0	2,5	-19
<i>Thymus vulgaris</i>	24 ±1.46 ^b	16±1.94	9±0.64	3±1.75	0.0	2,5	-12
<i>Szygium aromaticum</i>	27±1.19 ^{ab}	18±0.9	11±1.92	3.5±2.1	0.5±1.4	1	-6
Streptomycin	31±1.7 ^a	22±2.00	17±2.01	10±2.43	6±1.7	1	-
Control (water)	0.0 ^h	0.0	0.0	0.0	0.0	-	-
Control (water)	0.0 ^k	0.0	0.0	0.0	0.0	-	-

*The same letters next to the mean values (n = 3) in the column indicate that the difference between the applications is not statistically significant (Tukey multiple comparison Test, P≤0.05)

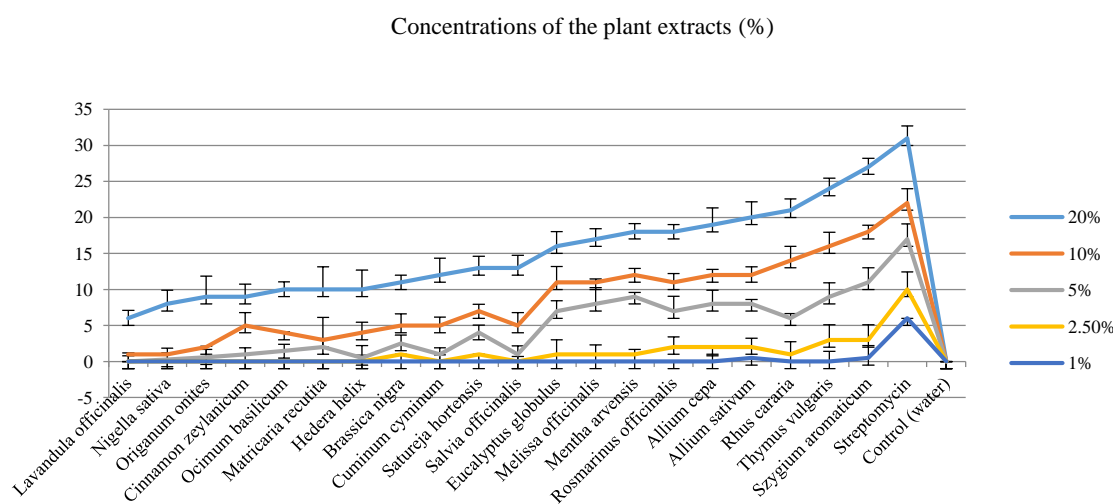


Figure 1. Antibacterial activities of some medicinal and aromatic plant extracts on *Erwinia amylovora*

In vivo Assays

In *in vitro* studies, three plants extracts, which were determined as the most effective 3 plant extracts, were tested on apple cv. Gala and pear cv. Santa Maria seedlings. In parallel with the *in vitro* findings, the lowest percent disease severity following streptomycin was obtained with the application of *S. aromaticum* (26.2% - 41.8%) and *T. vulgaris* (29.2% - 42.2%) extracts. They were followed by *R. cararia* (32.8% - 51.7%). In this work, it was determined that *S. aromaticum* and *T. vulgaris* extracts had the highest efficacies against fire blight disease in Central Anatolia conditions (Table 2 and 3, Figure 2 and 3).

Table 2. Disease severity and the efficacy of medicinal and aromatic plant extracts against fire blight disease on apple cv. Gala seedlings *in vivo*

Applications	Disease Severity (%)	Efficacy of Plant Extracts (%)
<i>Rhus cararia</i>	32.8±1.11*	59.70 ^d
<i>Thymus vulgaris</i>	29.2±3.52	64.12 ^c
<i>Szygium aromaticum</i>	26.2±2.34	67.81 ^b
Streptomycin	0.9±1.73	91.29 ^a
Control (water)	81.4±2.40	-

*The same letters next to the mean values (n = 3) in the column indicate that the difference between the applications is not statistically significant (Tukey multiple comp. test, P≤0.05)

Table 3. Disease severity and the efficacy of medicinal and aromatic plant extracts against fire blight disease on pear cv. Santa Maria seedlings *in vivo*

Applications	Disease Severity (%)	Efficacy of Plant Extracts (%)
<i>Rhus cararia</i>	51.7±2.11	40.02 ^{*c}
<i>Szygium aromaticum</i>	41.8±1.09	51.50 ^b
<i>Thymus vulgaris</i>	42.2±3.2	51.04 ^b
Streptomycin	2.3±1.18	97.33 ^a
Control (water)	86.2±2.19	-

*The same letters next to the mean values (n = 3) in the column indicate that the difference between the applications is not statistically significant (Tukey multiple comp. test, P≤0.05)

Re-isolation and identification of the pathogen

Isolated representative bacterial strains from apple and pear plants were Gram negative, rod-shaped, mucoid, fermentative, positive for gelatin hydrolysis, acetoin production and levan formation and showed no growth at 36 °C. The strains were negative for esculin hydrolysis, oxidase, indole, catalase, urease, arginine dihydrolase, reduction of nitrate, and acid production from inositol and lactose. PCR products gave 1 kb specific DNA fragments. According to the results of biochemical and molecular tests, the causal agent was identified as *E. amylovora*. The pathogen was not isolated from the control plants.

Discussion

The control of plant pathogenic bacteria is mainly achieved by the use of antibiotics and copper compounds which can result in toxicity to environment and humans, as well as accumulation in nature. In addition, the bacteria commonly acquire resistance to bactericides as a result of their continual uses. Plant derived chemicals have no

known negative impact on human health and on the environment. Thus, plant natural products are used for the development of new agrochemicals, especially biopesticides (Mosch et al., 1993; Vasinauskienė et al., 2006; Dorman and Deans, 2000; Isman, 2006; Shaheen, 2010; Dubey et al., 2011; Savoia, 2012; Gwinn, 2018).

Controlling of fire blight disease with plant extracts appears promising considering to the lack of effective chemicals to copper compounds and no commercial resistant cultivars. Thus, their potential use in agriculture as alternatives to or in combination with copper compounds are suggested in fire blight control (Smith, 1991; Mosch et al., 1993; Dorman and Deans, 2000; Psallidas and Tsianthos, 2000; Vanneste et al., 2005; Chiriak and Ulea, 2012; Savoia, 2012; Gwinn, 2018).

According to El-Hamid (2016), plant extracts have an ability to inhibit the expression of specific induced gene (s) during bacterial communication. They are very important tool for the management of bacterial pathogenesis through modulation of virulence genes. The antibacterial products obtained from plant extracts reduce bacterial infection by inhibiting the quorum sensing (QS) mechanism and biofilm formation in many Gram negative and positive bacteria. Flavonoids, alkaloids, terpenes, phenolics, polyphenols and coumarins in the structure of plant extracts act on bacteria by these mechanisms (Savoia, 2011; Vieira et al, 2017). In addition, plant extracts are important natural chemicals that stimulate systemic acquired resistance (SAR) in plants, are toxicologically safe, can be applied in low amounts, do not create resistant races in pathogens, and can be effective in integrated management programs for many diseases and pests (Baysal et al., 2003).

Chudasama and Thaker (2013) screened the antibacterial effects of many aromatic plant oil extracts against *Xanthomonas campestris* pv. *citri* revealed that both *S. aromaticum* and *T. vulgaris* had strong zones of inhibition 24.33 and 23.66 mm, respectively. Likewise, the results of Benchouikh et al (2016) antibacterial activity of *S. aromaticum* extract on *X. a.* pv. *phaseolicola* was strongly agree with this study. The methanol extracts of *S. aromaticum* showed high potential antibacterial activity against *Bacillus cereus*, *Salmonella typhi* and *Staphylococcus aureus* (Ibrahim and Abu-Salem 2014).

Sharma et al. (2014) and Benchouikh et al. (2016) reported that phenolic acids as a secondary plant metabolite such as eugenol and eugenyl acetate, which is found in *S. aromaticum*, have antibacterial effect. Similarly, Soltani and Aliabadi (2013) indicate that the antibacterial activity of the extracts is related to a number of flavonoids and phenolic compounds which are in pure form.

The antimicrobial activities of thyme essential oil are mostly attributed to the active mono terpene phenolic components. These terpene phenols interact with the amine and hydroxylamine groups of the proteins on bacterial membrane altering their permeability and resulting in death of bacteria (Scortichini and Rossi, 1991; Faleiro et al., 2003; Chizzola et al., 2008; Saraç and Uğur, 2008). *T. vulgaris* (Kokoskova and Pavela, 2007; Karamiosboos et al., 2010), which were tested against *E. amylovora* *in vitro*, has important effects on pathogen suppression and *T. vulgaris* essential oil content significantly prevented the development of the pathogen more than its extract composition.

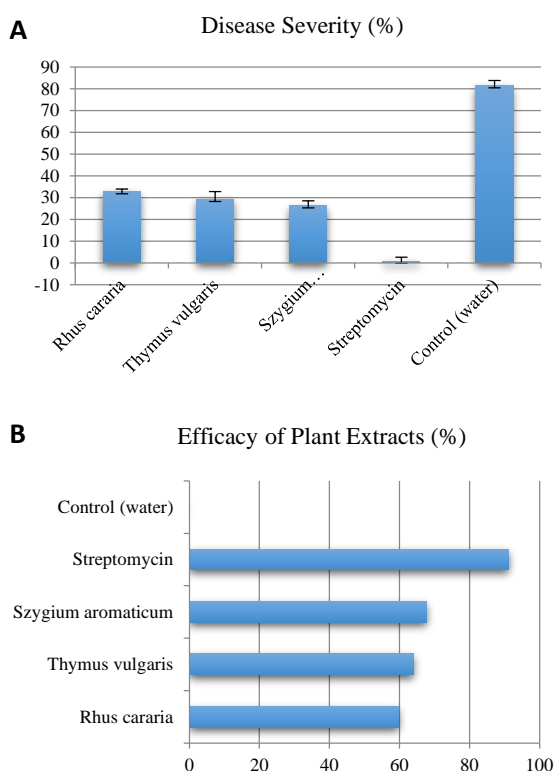


Figure 2. Effects of some medicinal and aromatic plants against *E. amylovora* on apple cv. Gala seedlings A. Disease severity (%) and B. Efficacy of plant extracts (%)

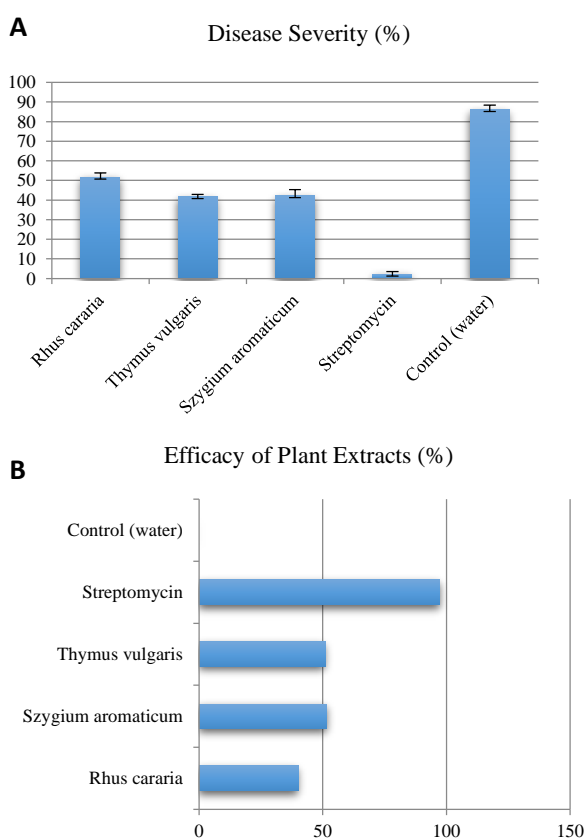


Figure 3. Effects of some medicinal and aromatic plants against *E. amylovora* on pear cv. Santa Maria seedlings A. Disease severity (%) and B. Efficacy of plant extracts (%)

Fire blight applications belonging to *Rhus coriaria*, which is effective in the second place in this study, have not been encountered in our country. The *R. coriaria* extract successfully reduced the fire blight severity caused by virulent isolate in our studies. Some researchers indicated that the *in vivo* antibacterial activity of the plant continued to be effective for at least 15 days, reducing treatments involving copper compounds. The characterization of the *R. coriaria* compounds and understanding of their active mechanisms might contribute to a better utilization of them either alone or in combination with other natural extracts. Phytochemicals in *R. coriaria* are being used as antibacterial and antiviral due to their contents of ellagic acid, gallic acid, isoquercitrin, myricitrin, myricetin, quercetin, quercitrin and tannic acid (Iauk et al., 1998; Ertürk, 2010; Abu-Reidah and Mohammed, 2014).

In addition, while the effectiveness of thymus on plant pathogens is well known, the situation of *R. coriaria* in preventing of fire blight should be re-tested with those grown in different regions and those obtained by different extraction methods.

Many organic solvents such as benzene, chloroform, ethyl acetate or methanole can be used for extraction of medicinal and aromatic plants. Comparing with water, methanol extraction of these plants was found highly successful (pre-experiments in this study), because of this reason, we used methanol extraction method. However, advantages of water extraction are their easily preparation, eco-friendly method and low cost.

The possible reasons why the effect levels of some plant extracts used in our study were determined as low, it is thought that is varied depending on the influence of one or more factors; these are geographical area where these medicinal and aromatic plants were grown, the growing conditions of that year, the presence of the subspecies of the plant, the harvest time, the way of harvest, the storage conditions, the methods used to obtain the extract and the extract content (Soylu et al., 2010).

To our knowledge, medicinal and aromatic plant extracts were tested in planta (on apple cv. Gala and pear cv. Santa Maria) the first time in our country and they proved useful for effective biocontrol of *E. amylovora*. No negative (phytotoxic) effects were recorded on the apple and pear seedlings tested.

Investigations are being carried out to understand more thoroughly their role in antibacterial efficacy of herbal extracts against different bacterial plant pathogens, as well as their large-scale use in disease management. We agree that plant essential oil composition is more effective, low cost and easier to use in the field on the pathogens however it is needed applicable new formulations especially for field treatments. In further researches, it should be investigated differences of Gram negative and positive bacteria both thyme, sumac and clove treatments and, interactions between host and defence enzymes and pathogenesis related proteins in the extract treatments.

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