

**STUDY REGARDING EFFECTIVENESS OF SOME  
PLANT EXTRACTS AND DIFFERENT PESTICIDES  
AGAINST AN *ERWINIA AMYLOVORA* (BURRILL.)  
WINSLOW *ET AL.* STRAIN ISOLATED FROM QUINCE**

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**ABSTRACT.** The interest in management fire blight prevention and control increased considerably in last years due to pathogen spread almost throughout the country. The objective of this paper focuses on testing of some plant extracts such as *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula*, *Galium verum* and different pesticides against an *Erwinia amylovora* strain isolated from quince damaged samples. In this study, the biological material was represented by quince shoots, harvested from pomological collection of "Vasile Adamachi" farm, which belongs to University of Agricultural Science and Veterinary Medicine Iași. The research were performed in the Phytopathology laboratory. Following the experiment performed note that among the eight plant extracts a good antibacterial activity has *Pelargonium odoratissimum*, *Ocimum basilicum* and *Salvia officinalis* and of the three pesticides, Alcupral 50 PU. Contrary to expectations worst results were obtained after testing Aliette 80 WG and *Hedera helix* products.

The results were estimated statistically, by performing ANOVA test.

**Key words:** Fire blight; Plant extracts; *Hedera helix*, Alcupral 50 PU.

**REZUMAT.** Studiul privind eficacitatea unor extracte din plante și pesticide asupra unei sușe de *Erwinia amylovora* (Burrill.) Winslow *et al.*, izolată de pe gutui. Interesul pentru managementul prevenirii și combaterii focului bacterian al rozaceelor a crescut considerabil în ultimii ani, ca urmare a răspândirii patogenului pe aproape întreg teritoriul țării noastre. Obiectivul acestei lucrări are în vedere testarea unor extracte din plante precum *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula*, *Galium verum* și a unor molecule active, asupra unei sușe de *Erwinia amylovora*, izolată din lăstarii de gutui, ce prezentau simptome tipice atacului de foc bacterian. Materialul luat în studiu a fost recoltat din ferma "Vasile Adamachi" din cadrul Universității de Științe Agricole și Medicină Veterinară Iași, iar cercetările

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s-au efectuat în laboratorul de cercetare al disciplinei de Fitopatologie. În urma experimentului efectuat se observă că, dintre cele opt extracte de plante, cea mai bună activitate antibacteriană o prezintă *Pelargonium odoratissimum*, *Ocimum basilicum* și *Salvia officinalis*, iar dintre cele trei pesticide, Alcupral 50 PU. Contrar așteptărilor, cele mai slabe rezultate s-au obținut în urma testării produselor Aliette 80 WG și *Hedera helix*. Rezultatele au fost interpretate statistic, folosind testul ANOVA.

**Cuvinte cheie:** focul bacterian; extracte din plante; *Hedera helix*; Alcupral 50 PU.

## INTRODUCTION

This disease was first reported in Romania in 1992, in two counties from the Southeast region: Pitești (Mărăcineni) and Brăila (Severin, 1996). Fire blight disease spread rapidly, in 1993 and since has been reported in nine counties (Constantinescu *et al.*, 1994) and in 2000 all counties across Romania have reported the presence of the disease (Vlad, 2003). Most economically relevant apple and pear cultivars are highly susceptible to fire blight, a risk even more intensified in modern high-density orchards (Longstroth, 2001). *Erwinia amylovora* is a pathogen that affects plants from the *Rosaceae* family and has a wide host range. Fire blight has been described in around 200 species included in 40 rosaceous genera (van der Zwet and Keil, 1979). Among the four subfamilies of the *Rosaceae*, *Maloideae* (syn. *Pomoideae*), *Rosoideae*, *Amygdaloideae* (syn. *Prunoideae*) and *Spireoideae* only in

the last fire blight has not been yet described. Chemical control relies upon the use of antibiotics (such as streptomycin) and copper compounds which prevent bacterial multiplication and further infection. Unfortunately, the antibiotics have lead to the selection of resistant bacterial populations and therefore their use is strictly limited or even forbidden in a number of countries (Cesbron *et al.*, 2000). To control fire blight, many researchers have tested various natural components such as plant extracts and essential oils to determine their antibacterial activity against the pathogen *Erwinia amylovora* since 1989 in Europe, and later also to check ability to induce resistance.

Aim of this study was to test *in vitro* antibacterial activity of some plant extracts compared to three different pesticides against E.A. - A.G. strain, isolated from pomological collection of "Vasile Adamachi" farm.

## MATERIAL AND METHOD

From quince samples with typical fire blight symptoms, harvested from pomological collection of "Vasile Adamachi" farm, we isolated after performing the patogenicity test on immature pear fruits (Billing *et al.*, 1960), a strain of *Erwinia amylovora* called E.A.- A.G.

In order to obtain plant extracts, flowers, leaves and stem portions of *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula* and *Galium verum* were used. These were placed in

## SUSCEPTIBILITY OF *ERWINIA AMYLOVORA* STRAIN TO PLANT EXTRACTS AND PESTICIDES

glass jars with grain alcohol 40%. Dishes were kept at 28° C and dark for two weeks. After 14 days, the plant extracts obtained were stored at 4° C and darkness. Chemical control was performed using three different active substances: fosetyl aluminum (Aliette 80 WG), copper hydroxide (Champ 77 WG) and copper oxychloride (Alcupral 50 PU).

**Culture media and growth conditions.** Bacteria was grown at 28°C on Nutrient Sucrose Agar (Billing *et al.*, 1961) and King's B (King *et al.*, 1954) medium and examined for fluorescence under ultraviolet light. Nutrient Sucrose Agar: NaCl 5 g, beef extract 1 g, peptone 5 g, agar 30 g, yeast extract 2 g, sucrose 50 g, NaOH 6 mL, distilled water 1000 mL, pH adjusted to 7.2 and sterilised by autoclaving at 121° for 20' (Billing *et al.*, 1961). King B medium: peptone 20g, glycerol 10 mL, K<sub>2</sub>HPO<sub>4</sub> 1,5g, MgSO<sub>4</sub> · 7 H<sub>2</sub>O 1,5 g, agar 15 g, distilled water 1000 mL, pH adjusted to 7.0 – 7.2 and sterilised by autoclaving at 121° for 20' (King *et al.*, 1954).

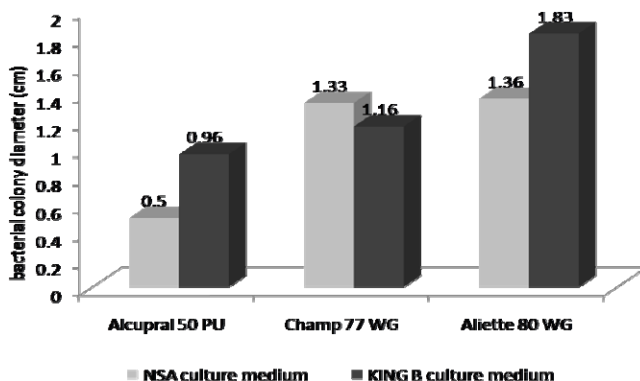
Microbiological media plates were prepared using Masterclave 09 plate maker and an aliquot portion of 16mL of media was poured using APS 320 automated Petri plate filler (AES). In each Petri dish was added 1mL of each plant extract and pesticide used and mixed together. All Petri dishes were sown with E.A. – A.G., *Erwinia amylovora* strain and than incubated at 28°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters. The results were estimated statistically, by performing ANOVA test.

## RESULTS AND DISCUSSION

Bacterial colonies outlines were recorded after their appearance in

Petri dishes, each 12 hours using different color markers. *Figure 1* presents data concerning bacterial colony diameter after 42 hours, because then no differences were recorded. Analyzing the figure below, we can see differences between the bacterial colonies diameter on NSA and King B medium mixed with the same products.

Although fire blight has been known for more than 200 years, most, if not all developed bactericides tested against this disease have shown no satisfactory and reliable to be used in the spray methodology, recommended for field application. There are many reasons behind, the main one being the nature of the disease and lack of effective, commercially available, environmentally safe and non-phytotoxic systemic bactericides (Psallidas and Tsiantos, 2000). Of the three active molecules tested *in vitro* against E.A. – A.G. strain, copper oxychloride (Alcupral 50 PU) was able to inhibit the bacterial activity. This is confirmed not only by reduced diameter of bacterial colonies but also of their color, which is dark brown. The effectiveness of copper against various pathogens is attributed to the availability of copper ions that inactivate many different enzymes and other proteins essential to vital cell membrane function. Under the same conditions, copper hydroxide (Champ 77 WG) showed a weak antibacterial activity comparing to copper oxychloride (Alcupral 50 PU).



**Figure 1 - The diameter of bacterial colony formed by E.A. - A.G. strain on NSA and King B culture medium mixed with pesticides**

Fosetyl-aluminum is an acidic systemic fungicide marketed under the name Aliette and used primarily against different species of the genus *Phytophthora*. However, because of its systemic property, fosetyl aluminum was thought to be a good bactericide against fire blight. The results of experiments conducted in both the USA and Europe showed that its effectiveness was inconsistent. Norelli and Aldwinckle (1993) reported that the use of Aliette gave poor control of fire blight under both high or low inoculum pressure and it failed to control blossom blight in spring when applied the previous autumn. Following the experiment performed *in vitro*, we found low efficiency of this product to destroy the bacterium *Erwinia amylovora*.

Figure 2 presents the results on the effectiveness of antibacterial plant extracts tested. On both culture media mixed with the extract obtained from *Pelargonium odoratissimum*, E.A. -

A.G. strain has formed small colonies. Mosch *et al.* (1989) also tested, the extract of *Pelargonium odoratissimum in vitro* and the results were satisfactory. Antibactericid effect of this extract was confirmed by the results in control of *Erwinia amylovora* strain isolated from pear shoots (Chiriac and Ulea, 2012). On NSA culture medium, *Pelargonium odoratissimum*, *Ocimum basilicum* and *Salvia officinalis*, showed a very good antibacterial activity.

Analyzing the data in the figure above we see that the extracts obtained from *Pelargonium odoratissimum*, *Ocimum basilicum* and *Salvia officinalis* showed antibacterial activity better than, that obtained from *Hedera helix*.

Six of the 11 products tested *in vitro* showed a lower bacterial colony diameter on NSA medium, compared with those of the same environmental conditions on King B.

SUSCEPTIBILITY OF *ERWINIA AMYLOVORA* STRAIN TO PLANT EXTRACTS AND PESTICIDES

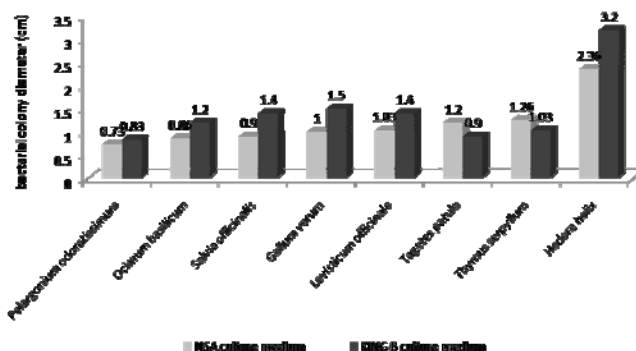


Figure 2 - The diameter of bacterial colony formed by E.A. - A.G. strain on NSA and King B culture medium mixed with plant extracts

Table 1 - The results of ANOVA test

Anova: Single Factor						
SUMMARY						
Groups	Count	Sum	Average	Variance		
Alcupral 50 PU	2	1.46	0.73	0.1058		
<i>Pelargonium odoratissimum</i>	2	1.56	0.78	0.005		
<i>Ocimum basilicum</i>	2	2.06	1.03	0.0578		
<i>Salvia officinalis</i>	2	2.3	1.15	0.125		
<i>Galium verum</i>	2	2.5	1.25	0.125		
<i>Levisticum officinale</i>	2	2.43	1.215	0.06845		
<i>Tagetes patula</i>	2	2.1	1.05	0.045		
<i>Thymus serpyllum</i>	2	2.29	1.145	0.02645		
Champ 77 WG	2	2.49	1.245	0.01445		
Aliette 80 WG	2	3.19	1.595	0.11045		
<i>Hedera helix</i>	2	5.56	2.78	0.3528		
ANOVA						
Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	6.115	10	0.6115	6.491507	0.00237	2.853625
Within Groups	1.0362	11	0.0942			
Total	7.1512	21				

The results were estimated statistically, by performing ANOVA test in Microsoft Office Excel 2007 (Table 1). The p-value tells us that the lowest significance level (0.01) attainable is only 0.00237 and  $F > F_{crit.}$ , so in this situation we are able to

reject the null hypothesis. Therefore, there is very strong evidence to suggest that the means are not all equal. According to statistics the products tested *in vitro* show significant differences to inhibit E.A. – A.G. strain.

## CONCLUSIONS

The highest antibacterial efficiency was found after testing copper oxychloride (Alcupral 50 PU) on both Nutrient Sucrose Agar and King B culture media.

Following the experiment performed *in vitro*, *Pelargonium odoratissimum*, *Ocimum basilicum* and *Salvia officinalis*, showed good antibacterial activity.

Six of the 11 products tested *in vitro* showed a lower bacterial colony diameter on NSA medium, compared with those of the same environmental conditions on King B.

The weak antibacterial activity was recorded for the extract obtained from *Hedera helix* and Aliette 80 WG pesticide.

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