

## **IN VITRO SUSCEPTIBILITY OF *ERWINIA AMYLOVORA* (BURRILL) WINSLOW *ET AL.* STRAIN ISOLATED FROM PEAR TO SEVERAL PLANT EXTRACTS AND DIFFERENT PESTICIDES**

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**ABSTRACT.** In less than six months after infection, *Erwinia amylovora* may completely destroy a pome tree. This paper aims to increase awareness of the potential for developing plant protection strategies based on natural products. Since the damage caused by this bacterium are unrecoverable, we tested in vitro the effectiveness of a several plant extracts such as *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula*, *Galium verum* and some pesticide products, on a strain of *Erwinia amylovora* isolated from the pomological collection of "Vasile Adamachi" farm, the University of Agricultural Sciences and Veterinary Medicine, Iași. The strain was isolated from damaged pear shoots, after performing pathogenicity test on immature pear fruits. Following the experiment conducted, copper oxychloride (Alcupral 50 PU) and *Pelargonium odoratissimum* showed a very good antibacterial activity against EA - A.P. strain.

**Key words:** *Erwinia amylovora*, plant extracts, pesticide.

**REZUMAT.** Sensibilitatea unei sușe de *Erwinia amylovora* (Burrill) Winslow *et al.* izolată de pe păr în urma testării *in vitro* a unor extracte din plante și diferite pesticide. Bacteria *Erwinia amylovora* poate distruge un pom, în totalitate, în mai puțin de șase luni de la realizarea infecției. Această lucrare are în vedere dezvoltarea unor strategii de protecție a plantelor, bazate pe produse naturale. Întrucât pagubele produse de această bacterie sunt irecuperabile, ne propunem prin acest studiu să testăm, *in vitro*, o serie de extracte de plante, precum *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula*, *Galium verum*, și unele produse pesticide, pentru a verifica eficacitatea acestora asupra unei sușe de *Erwinia amylovora*, izolată din colecția pomologică a fermei "Vasile Adamachi", din cadrul Universității de Științe Agricole și Medicină Veterinară Iași. Sușa a fost izolată din ramurile de păr

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atacate, în urma testului de patogenitate, realizat pe fructele de pere verzi. Oxiclorura de cupru (Alcupral 50 PU) și *Pelargonium odoratissimum* au prezentat, în urma experimentului efectuat, cea mai bună activitate antibacteriană împotriva sușei E.A. - A.P.

**Cuvinte cheie:** *Erwinia amylovora*; extracte de plante; pesticide.

## INTRODUCTION

Fire blight, caused by the bacterium *Erwinia amylovora* (Burrill) Winslow *et al.* and reported in more than 40 countries around the world, is a very serious and destructive disease of pome fruits and many ornamental plants from the *Rosaceae* family (Van der Zwet and Beer, 1995). There is no single control measure for fire blight that will totally eradicate the disease, provide an absolute cure, or fully protect an orchard. However, fire blight damage can be kept to a minimum (Aldwinckle *et al.*, 1998).

A large number of chemicals have been tested against fire blight disease: cooper compounds, antibiotics, carbamates and miscellaneous compounds. Two groups of chemicals that have the most important role in controlling the disease on apples and pears trees are cooper compounds and antibiotics. The main disadvantage of cooper compounds is their phytotoxicity on host plants, especially pears (Măruțescu *et al.*, 2009). Copper compounds, widely utilized against fire blight from the beginning of the

last century (Van der Zwet and Keil, 1979), are still employed in many countries, especially in the European Union, where antibiotic utilization is restricted (Anonymous, 1999). Their use is one of the most common methods for controlling bacterial plant diseases, but it has led many bacteria to develop different strategies against copper ions (Saxena *et al.*, 2002). During the last 20 years, biological control of fire blight has advanced from a subject of basic research to a component of an integrated disease control strategy in the United States of America, but later on after dissemination of the pathogen under European conditions (Johnson and Stockwell, 2000). Mosch *et al.* (1989), Scortichini and Rossi (1989), Scortichini and Rossi (1991), Mosch *et al.*, (1993), Mosch *et al.* (1996) have tested numerous plant extracts *in vitro* and *in vivo* against *Erwinia amylovora*. An alternative to the antibiotic streptomycin, several natural compounds, like plant extracts and etheric oils have been tested for their antibacterial activity against *Erwinia amylovora* since 1989 in Europe, and later on also for their induced resistance response.

The objective of this study was to find if the products tested against *Erwinia amylovora* are able to inhibit or eradicate the bacteria. The results obtained from this study may contribute to the development of new bio-control compounds as alternative strategies to protect fruit trees from fire blight disease.

## MATERIALS AND METHODS

From pear samples with typical fire blight symptoms, harvested from pomological collection of "Vasile Adamachi" farm, we isolated after performing the pathogenicity test on immature pear fruits, a strain of *Erwinia amylovora* called E.A.- A.P. The bacteria was grown on two different culture media. In order to obtain plant extracts, in August were collected eight species of plants: *Salvia officinalis*, *Thymus serpyllum*, *Pelargonium odoratissimum*, *Hedera helix*, *Ocimum basilicum*, *Levisticum officinale*, *Tagetes patula* and *Galium verum*. Flowers, leaves and pieces of stem were placed in glass jars with grain alcohol 40%. Dishes were kept warm and dark for two weeks. After 14 days, the plant extracts obtained were stored at low temperature 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 and King's B 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'. 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

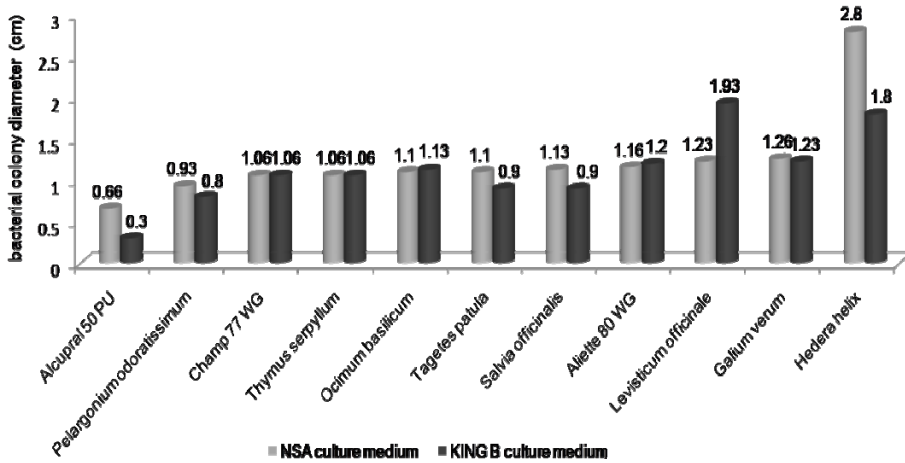
automated Petri plate filler (AES Laboratoire, France). In each Petri dish was added 1mL of each plant extract and pesticide used and mixed together. All Petri dishes were sown with E.A. – A.P. *Erwinia amylovora* strain and then incubated at 28°C. Visual observation were made regarding the colony form, size and color each 12 hours. The results were estimated statistically, by performing T test.

## RESULTS AND DISCUSSION

Petri plates were analyzed after about 18 hours, when it noticed the presence of bacteria on both culture media. The colony shape was marked using a different marker. After each 12 hours were carried out visual observations and measurements of bacterial colonies. *Figure 1* presents data concerning bacterial colony diameter after 42 hours, because then no differences were recorded.

Since 1900, copper compounds have been established as effective bactericides and have been used against fire blight on apples and pears (Van der Zwet and Keil, 1979). After conducting *in vitro* experiments significant differences were recorded between the two copper compounds.

After 42 hours, the colony grown on King B medium mixed with copper oxychloride (Alcupral 50 PU) had a diameter of 0.3 cm while the same culture medium mixed with copper hydroxide (Champ 77 WG) value recorded was 1.06 cm. Among the three pesticides tested the most effective product is Alcupral 50 PU, followed by Champ 77 WG and Aliette 80 WG.



**Figure 1 - The diameter of bacterial colony (*Erwinia amylovora*) on NSA and King B culture medium**

Considered a good product to control fire blight, Aliette 80 WG didn't show significant antibacterial activity, some plant extracts proved to be more effective. Fosetyl-aluminium, or its metabolite, phosphonic acid, probably acts directly on the target pathogen, slowing its growth, allowing the host plant's natural defence mechanisms to finally inhibit its growth. However, because of its systemic property, fosetyl-aluminium 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 (Psallidas and Tsiantos, 2000).

By definition, a natural or biogenic substance is either synthesized directly by a living organism or is derived from

substances of biogenic origin by chemical reactions occurring without human intervention; for example by decomposition of biological materials (Slusarenko *et al.*, 2008). These are obvious candidates to be considered for use in plant-protection strategies. Furthermore, many substances of natural origin which do not show direct antimicrobial activity might act as resistance inducers to prime systemic acquired resistance relying on the plant's own defences (Goellner and Conrath, 2007).

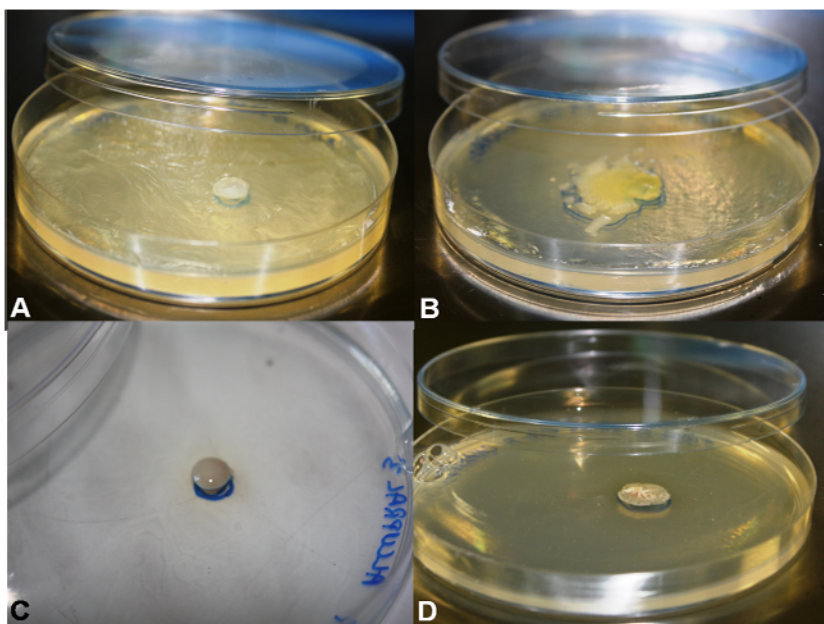
Most plant extracts tested against bacteria *Erwinia amylovora*, showed good antibacterial activity. Among the eight plant extracts, four were tested *in vitro* and *in vivo* by other researchers. Mosch *et al.* (1989, 1993 and 1996) found that *Salvia officinalis*, *Pelargonium*

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*odoratissimum*, *Hedera helix* showed good antibacterial activity and moderate antibacterial activity *Thymus serpyllum*. Following experiments conducted *in vitro*, *Pelargonium odoratissimum* managed to inhibit growth of bacteria on both culture media, so after 42 hours did not exceed 0.93 cm colony diameter on the NSA. After testing the extract obtained from *Hedera helix* significant differences were recorded. The products tested were able to

inhibit the activity of the bacteria and not to destroy completely the pathogen.

On King B culture medium, *Erwinia amylovora* colonies develop circular, type S (smooth), with sharp edges, white (Miller, 1984), and on NSA they are also circular, are slightly convex, type S and yellow white (Billing, 1974). On both culture media mixed with several products, bacterial colonies presents different shape, size and color (Fig. 2).



**Figure 2 - Different color and shape of *Erwinia amylovora* bacterial colony on King B culture medium mixed with: A - *Pelargonium odoratissimum*, B - *Hedera helix*, C - copper oxychloride (Aicupral 50 PU), D - copper hydroxide (Champ 77 WG)**

As a result of the T test, we see that, on different culture media mixed with the same product *Erwinia amylovora* record almost the same values (Table 1). The only

differences are reported for *Hedera helix* and *Levisticum officinale* plant extracts.

Moreover, additional studies on combination of bioproducts with each

other or with other compounds should be promoted for enhancing the efficacy against this dangerous disease. These aspect should be an

important objective for further research on development of new and efficient biocontrol methods against fire blight (Zeller, 2006).

**Table 1 - The results of T- Test**

<b>t-Test: Two-Sample Assuming Equal Variances</b>	<b>Variable 1</b>	<b>Variable 2</b>
Mean	1.226363636	1.119090909
Variance	0.298705455	0.201589091
Observations	11	11
Pooled Variance	0.250147273	
Hypothesized Mean Difference	0	
df	20	
t Stat	0.503005554	
P(T<=t) one-tail	0.310227386	
t Critical one-tail	1.724718218	
P(T<=t) two-tail	0.620454771	
t Critical two-tail	2.085963441	

## CONCLUSIONS

Although it is considered an effective bactericide against *Erwinia amylovora*, after testing *in vitro* the *Aliette 80 WG* pesticide, the results showed no significant antibacterial activity, some plant extracts proved to be more effective.

Among the three pesticides tested *in vitro*, copper oxychloride (Alcupral 50 PU) gave the best results, bacterial colonies sizes on King B culture medium being only 0.3 cm in diameter.

The larger bacterial colonies were recorded on NSA culture medium mixed with the extract obtained from *Hedera helix*.

Between the eight plant extracts tested *in vitro* the best antibacterial activity was submitted by *Pelargonium odoratissimum*.

*Salvia officinalis* and *Tagetes patula* also inhibits growth of bacteria *Erwinia amylovora*, the values recorded are almost the same.

## REFERENCES

- Aldwinckle H.S., Norelli J.L., Momol M.T., 1998** - *Erwinia amylovora* can move downward inside apparently healthy branches and trunk from scion infections in blossoms and leafy shoots into the rootstock, IDFTA Compact Fruit Tree, Vol. 31, No. 4.
- Anonymous, 1999** - Amounts of antimicrobial used, p. 44–45. In Opinion of the scientific steering committee on antimicrobial resistance. Unit B3—Management of scientific committees II. Directorate General XXIV Consumer Policy and Consumer Health Protection. European Commission, Brussels, Belgium.

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- Billing E., 1974** – The effect of temperature on the growth of the fire blight pathogen, *Erwinia amylovora*, J., Appl. Bacteriology 37: 643 – 648.
- Goellner K., Conrath U., 2007** - Priming: It's all the world to induced disease resistance EJPP this issue.
- Johnson K.B., Stockwell V.O., 2000** - Biological Control of Fire Blight. In Vanneste J.L. (Ed). Fire blight: the Disease and its Causative Agent, *Erwinia amylovora*, CABI Publishing Wallingford, pp: 319-337.
- King E.O., Ward M., Raney D.E., 1954** - Two simple media for the demonstration of pyocyanin and fluorescein, J. Lab. Clin. Med. 44: 301-307.
- Măruțescu L., Saviuc C, Oprea E., Savu B., Bucur M., Stanciu Gh., Chifiriuc M. C., Lazăr V., 2009** - In vitro susceptibility of *Erwinia amylovora* (Burrill) Winslow *et al.* to *Citrus maxima* essential oil, Roum Arch Microbiol Immunol., 68(4):223-7.
- Miller H.J., 1984** - *Erwinia amylovora* detection and its significance in survival studies. *Acta Hort.* 151:63-66.
- Mosch J., Klingauf F., Zeller W., 1989** - On the effect of plant extracts against fireblight (*Erwinia amylovora*). *Acta Horticulturae* 273, 355–361.
- Mosch J., Mende A., Zeller W., Rieck M., Ullrich W., 1993** - Plant extracts with a resistance induction effect against fireblight (*Erwinia amylovora*). *Acta Horticulturae* 338, 389–395.
- Mosch J., Zeller W., Rieck M., Ullrich W., 1996** - Further studies on plant extracts with a resistance induction effect against *Erwinia amylovora*, *Acta Horticulturae* 411, 361–366.
- Psallidas P. G., Tsiantos J., 2000** - Chemical Control of Fire Blight In Vanneste J.L. (Ed). Fire blight: the Disease and its Causative Agent, *Erwinia amylovora*, CABI Publishing Wallingford, pp: 202, 214, 227.
- Saxena D., Joshi N., Srivastava S., 2002** - Mechanism of copper resistance in a copper mine isolate *Pseudomonas putida* strain S4. *Curr. Microbiol.* 45:410–414.
- Scortichini M., Rossi M.P., 1989** - *In vitro* activity of some essential oils toward *Erwinia amylovora* (Burrill) Winslow *et al.* *Acta Phytopathologia et Entomologia Hungarica* 4, 423–431.
- Scortichini M., Rossi M.P., 1991** - Preliminary *in vitro* evaluation of the anti-microbial activity of terpene and terpenoids towards *Erwinia amylovora* (Burrill) Winslow *et al.* *Journal of Applied Bacteriology* 71, 109–112.
- Slusarenko A.J., Patel A., Portz Daniela, 2008** - Control of plant diseases by natural products: Allicin from garlic as a case study, *Eur J Plant Pathol* 121:313–322.
- Van der Zwet T., Keil H. L., 1979** – A Bacterial Disease of Rosaceous Plant, Agriculture Handbook no. 510, United States Department of Agriculture, Washington .D.C.
- Van der Zwet T., Beer S.V., 1995** - Fire blight - its nature, prevention, and control. A practical guide to integrated disease management. USDA agriculture information bulletin no. 631. U.S. Department of Agriculture, Washington, D.C.
- Zeller W., 2006** - Status of biocontrol methods against fire blight, *Phytopathol. Pol.* 39: 71–78.