ANTIBACTERIAL ACTIVITY OF SOME PLANT EXTRACTS AND DIFFERENT PESTICIDES AGAINST AN *ERWINIA AMYLOVORA* (BURRILL.) WINSLOW ET AL. STRAIN ISOLATED FROM A NURSERY STOCK

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Abstract. *Fire blight is considered the most damaging disease of seed trees and the pathogen is one of the most studied. Since the damage caused by Erwinia amylovora is large and irrecoverable we intend to control the bacteria. The experiment was carried out at Phytopathology Laboratory of the University of Agricultural Sciences and Veterinary Medicine of Iasi. The bacteria was isolated from damaged quince shoots harvested from a nursery stock located in Sârca, Iasi, after performing the patogenicity test in early june. Two culture media were used to test the effectiveness of some products to control the pathogen, NSA (Nutrient Sucrose Agar) and King B. In order to destroy the bacteria eleven products were used, three pesticides: Alcupral 50 PU, Champ 77 WG, Aliette 80 WG and eight plant extracts: Salvia officinalis, Thymus serpyllum, Pelargonium odoratissimum, Hedera helix, Ocimum basilicum, Levisticum officinale, Tagetes patula and Galium verum. All Petri dishes were sown with E.A. – S.G. Erwinia amylovora strain and than incubated at 28°C. Antimicrobial activity was assessed by measuring the growth inhibition zones diameters. Out of the eight plant extracts five presented a good antibacterial activity. Erwinia amylovora formed colonies with a diameter less than 1 cm on King B medium mixed with Tagetes patula, Galium verum, Ocimum basilicum, Pelargonium odoratissimum, Alcupral 50 PU and Salvia officinalis. The shape, color and size of bacterial colonies varies significantly. Extracts from Salvia officinalis, Hedera helix and Levisticum officinale, showed a weak and very weak antibacterial activity, since bacterial colonies formed by Erwinia amylovora had diameters between 1.13 and 2.73 cm. Diameter formed by Erwinia amylovora on both the culture media mixed with copper oxychloride (Alcupral 50 PU) and copper hydroxide (Champ 77 WG) was much higher than that recorded in mixed culture media with different plant extracts. From the three fungicides tested in vitro the lowest antibacterial activity was recorded on culture media mixed with Aliette 80 WG. The results were interpreted statistically using ANOVA test.*

Key words**:** *fire blight, bacteria, Alcupral 50 PU*

INTRODUCTION

Fire blight has been considered as undoubtedly the oldest most serious and perplexing bacterial disease of pomaceous fruit trees (VAN DER ZWET and KEIL, 1979). About this disease it has been aid that "there is probably no disease of fruit trees as thoroughly destructive as fire blight" (WAITE, 1985 in THOMSON, 2000). Fire blight poses significant problems both for nurserymen and for growers who purchase trees from nurseries where blight occurs. Since the pathogen can remain latent in symptomless tissues (MCMANUS and JONES, 1994; CREPEL *et al.*, 1995), its unintentional introduction into nursery fields through contaminated bud wood and into growers orchards in newly purchased, dormant trees can be especially troublesome (STEINER, 2000). Chemicals are applied either to eliminate or to inactivate plant-pathogenic bacteria before these bacteria succeed in penetrating the host tissues. They are also applied to render the plant surfaces unsuitable for the establishment of new infections (PSALLIDAS and TSIANTOS, 2000). In chemical control of fire blight, a large number of compounds have been

tested. Aim of this study was to test *in vitro* antibacterial activity of some plant extracts compared to three different pesticides against E.A. - S.G. strain, isolated from a nursery stock.

MATERIAL AND METHODS

From quince samples with typical fire blight symptoms, harvested from a nursery stock located in Iasi, we isolated after performing the patogenicity test on immature pear fruits (BILLING et al. 1960), a strain of *Erwinia amylovora* called E.A. - S.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 glass jars with grain alcohol 40%. Dishes were kept at 28º C and dark for two weeks. After 14 days, the plant extracts obtained were stored at 4º 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 and King's B (KING et al, 1954) medium and examinated for fluorescence under ultraviolet light. 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. – S.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 DISCUSSIONS

Bacterial colonies outlines were recorded after their appearance in Petri dishes, each 12 hours using different color markers. Analyzing the figure below, we can see differences between the bacterial colonies diameter on NSA and King B medium mixed with different pesticides.

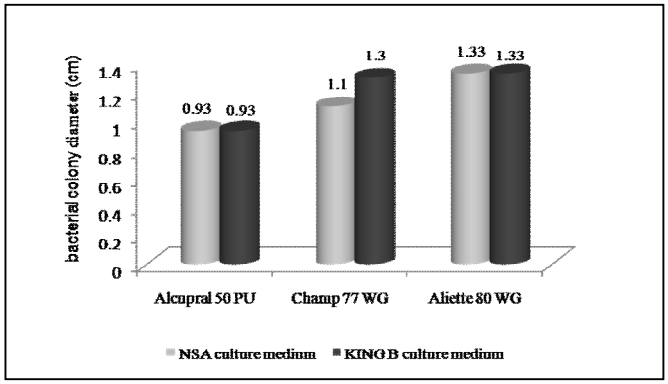

Figure 1: The diameter of bacterial colony formed by E.A. - S.G. strain on NSA and King B culture medium mixed with different pesticides

On both culture media, mixed with the same products, *Erwinia amylovora* recorded almost the same diameter of bacterial colony. The results of the experiment show that *Aliette 80 WG* has a low antibacterial activity (figure 1). Comparing the results obtained by different

investigators in controlling fire blight with fosetyl-Al with the results obtained from experiments against other bacterial diseases, it seems that there are difficulties in obtaining consistent results, not only from year to year but also from trial to trial. Because of this inconsistency, Chase (1993) does not recommend fosetyl-Al to be used for control of bacterial diseases on ornamental crops. *Aliette* (fosetyl-Al) was found completely ineffective.

The resistance of *Erwinia amylovora* to copper has not yet been detected; we cannot rule out that it will develop, since resistance to copper has been reported in other phytopathogenic bacteria. Copper derivatives have been extensively used to reduce losses due to *Erwinia* spp. in many crops and they have also been used to reduce fire blight of fruit trees (LOPER *et al*., 1991; SAYGILI and ÜSTÜN, 1996). Cooper compounds have been established as effective bactericides and have been used against fire blight on apple and pear since 1900 (VAN DER ZWET and KEIL, 1979). Copper products registered for use as fungicides or bactericides are low in phytotoxicity, have small particle sizes to optimize their availability, and have materials added to improve spreading and sticking. Most copper fungicides and bactericides contain at least 20-50% copper (NINOT *et al.*, 2002).

Diameter formed by *Erwinia amylovora* on both the culture media mixed with copper oxychloride (*Alcupral 50 PU*) and copper hydroxide (*Champ 77 WG*) was much higher than that recorded in mixed culture media with different plant extracts. The diameter of bacterial colonies formed by *Erwinia amylovora* strain on NSA and King B culture media mixed with different plant extracts are presented in figure 2.

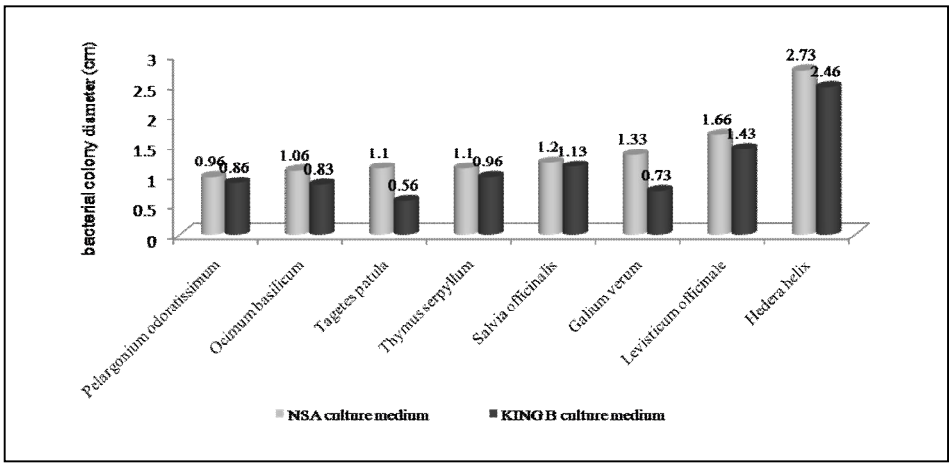

Figure 2: The diameter of bacterial colony formed by E.A. - S.G. strain on NSA and King B culture medium mixed with different plant extracts

On King B medium mixed with *Tagetes patula*, *Ocimum basilicum*, *Pelargonium odoratisimum*, *Thymus serphyllum* and *Galium verum*, the diameter of bacterial colonies is less than 1 cm. The shape, color and size of bacterial colonies vary significantly. Several researchers have tested various plant extracts to control *Erwinia amylovora*, but all this products used were able only to inhibit the activity of bacteria and not to destroy it completely. In 1991, SCORTICHINI and ROSSI found that *Origanum vulgare, Allium sativum* and *Satureja hortensis* extracts presented a very good antibacterial activity while *Salvia sclarea, Rosmarinus officinalis, Rosa canina, Pinus sylvestris* and *Mentha piperita* extracts cannot control the pathogen. Of the eight plant extracts tested against *Erwinia amylovora*, *Tagetes patula, Galium*

verum, Ocimum basilicum, Pelargonium odoratissimum and *Thymus serpyllum* showed a good antibacterial activity on King B medium and weak on NSA culture medium. The extract obtained from *Pelargonium odoratissimum* presented a good antibacterial activity both against the *Erwinia amylovora* strain isolated from quince shoots and the strain isolated from pear samples (CHIRIAC and ULEA, 2012). Extracts from *Salvia officinalis, Hedera helix* and *Levisticum officinale*, showed a weak and very weak antibacterial activity, since bacterial colonies formed by *Erwinia amylovora* had diameters between 1.13 and 2.73 cm.

SCORTICHINI and ROSSI, discover in 1989 that *Ocimum basilicum* extract do not present antibacterial activity, but after testing this plant extract *in vitro* on two different culture media we observe an antibacterial activity, specially on King B medium.

Although MOSCH et al., (1993), argues that the extract of *Hedera helix* is very effective both *in vitro* and *in vivo*, according to data recorded in figure 2, we see that from the eight tested plant extracts, *Hedera helix* has the weakest antibacterial activity.

The p-value tells us that the lowest significance level (0.01) attainable is only 0.000141and 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. – S.G. strain (table 1).

Table 1

The results of ANOVA test

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Alcupral 50 PU	2	1.86	0.93	0
Champ 77 WG	2	2.4	1.2	0.02
Aliette 80 WG	2	2.66	1.33	0
Pelargonium odoratissimum	2	1.82	0.91	0.005
Ocimum basilicum	2	1.89	0.945	0.02645
Tagetes patula	2	1.66	0.83	0.1458
Thymus serpyllum	2	2.06	1.03	0.0098
Salvia officinalis	2	2.33	1.165	0.00245
Galium verum	2	2.06	1.03	0.18
Levisticum officinale	2	3.09	1.545	0.02645
Hedera helix	2	5.19	2.595	0.03645

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.982327	10	0.498233	12.11441	0.000141	2.853625
Within Groups	0.4524	11	0.041127			
Total	5.434727	21				

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*, the extracts obtained from *Pelargonium odoratissimum* and *Ocimum basilicum* showed good antibacterial activity.

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

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

BIBLIOGRAPHY

1. BILLING E., CROSSE J.E., GARRETT C.U.E., 1960 - *Laboratory diagnosis of fire blight and bacterial blossom blight of pear*. Plant Pathol. 9: 19–25.
2. BILLING E., BAKER L.A.E., CROSS J.E., GARRETT C.M.E., 1961 - Characterization of English isolates of *Erwinia amylovora* (Burrill). Winslow *et. al*. *Journal of Applied Bacteriology* 24: 195-211.
3. CHASE A.R., 1993 - Efficacy of fosetyl-Al for control of some bacterial diseases on ornamentals. *Plant Disease* 77, 771–776.
4. CHIRIAC IRINA, ULEA E., 2012 - *In vitro* susceptibility of *Erwinia amylovora* (Burrill) Winslow *et al.* strain isolated from pear to several plant extracts and different pesticides, Cercetări Agronomice în Moldova, Vol. XLV , No. 1 (149) / 2012, pp. 69.
4. CREPEL C., GEENEN J., MAES M., 1995 - The latent survival of *Erwinia amylovora* in hibernating shoots. *Acta Horticulturae* 411, 21–25.
5. 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.
6. LOPER J.E., HENKELS M.D., ROBERTS R.G., GROVE G.G., WILLET M.J., AND SMITH, T.J. 1991 - Evaluation of streptomycin, oxytetracycline and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. *Plant Dis.* 75:287-290.
7. 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.
8. MCMANUS P.S., JONES A.L., 1994 - Role of wind driven rains, aerosols and contaminated budwood in incidence and spatial pattern of fire blight in an apple nursery. *Plant Disease* 78, 1059–1066.
9. NINOT A.; ALETÀ N., MORAGREGA C., MONTESINOS E., 2002 - Evaluation of a reduced copper spraying program to control bacterial blight of walnut. *Plant Dis.* 86:583-587.
10. 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.
11. SAYGILI H., ÜSTÜN, N. 1996 - Studies on effectiveness of some chemicals to fire blight pathogen *Erwinia amylovora* (Burril) Winslow *et al*. *Acta Hort.* 411:331-335.
12. 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.
13. 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.
14. SMITH T.J., 1991 - Evaluation of streptomycin, oxytetracycline and copper resistance of *Erwinia amylovora* isolated from pear orchards in Washington State. *Plant Dis.* 75:287-290.
15. STEINER P.W., 2000 - Integrated orchard and nursery management for the control of fire blight. Pp. 339-358.In: Vanneste, J.L. (ed.). Fire Blight. The Disease and its Causative Agent, *Erwinia amylovora*. ABIPublishing. Wallingford. UK.
16. THOMSON S.V., 2000 - Epidemiology of fire blight. p. 9-36. In: J.L. Vanneste (ed.), Fire Blight, The Disease and its Causative Agent, Wallingford, UK. *Erwinia amylovora*, CABI Publishing.
17. VAN DER ZWET T., KEIL H.L., 1979 - Fire blight- A bacterial disease of Rosaceous plants. Agriculture Handbook 510. US Department of Agriculture. Washington. pp. 200.