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EFFICACY OF CHEMICAL PRODUCTS AND EPIPHYTIC BACTERIA IN CONTROL OF FIRE BLIGHT (*ERWINIA AMYLOVORA*)

Abstract. In model tests carried out in a greenhouse on apple trees, it was shown that in conditions of high infection pressure of E. amylovora, the one-off, preventive use of Aliette 80WG during full bloom was effective in reducing blossom blight in the range of 60–70%. There was no significant increase in efficacy after using this product twice in comparison to single application. However, Aliette turned out to be ineffective in the protection of terminal shoots. High efficacy, both in protecting blossoms and apple shoots, was shown by a cooper-based formulation Miedzian 50WP, but it caused some phytotoxicity on flower petals. Also in the test on slices of pear fruitlets, this product caused significant ressetting. Overall, the highest efficacy among the different formulations and bacterial isolates tested was recorded when the pear fruitlet slices were treated with the bacterial isolate A15 (Pantoea agglomerans) (96%); other effective products were the antibiotic-based Kasumin 2L (94%) and Miedzian 50WP (89%). It is noteworthy that Controlphyt Cu, a foliar and soil fertilizer containing Cu, showed quite high efficacy – up to 71% when applied at the concentration of 0.4% and 41% at 0.2%.

Key words: fosetyl aluminium, copper, kasugamycin, biocontrol, apple, pear.

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Химия өнімдерінің және эпифит бактериялардың бактериялық күйік қоздырғышына (*Erwinia amylovora*) қарсы тиімділігі

Аңдатпа. Жылыжай жағдайында алма ағаштарға E. amylovora жоғары инфекциялық қысым жасау арқылы жүргізілген модельдік сынақтарда алдын ала бір рет Aliette 80WG қолдану, толық гүлдену кезеңінде бактериялық күйікті 60–70% дейін тиімді төмендеткені анықталды. Бір реттік қолданумен салыстырғанда, осы өнімді екінші рет пайдаланғаннан кейін тиімділіктің айтарлықтай артуы байқалмады. Алайда, Aliette терминалдық бұтақтарды қорғауда тиімсіз болды. Гүлдерді қорғауда да, алманың өркенін қорғауда да мыс купоросы негізіндегі Miedzian 50WP препараты жоғары тиімділікті көрсетті, бірақ ол гүлдердің жапырақтарында фитоуыттылықты тудырды. Сондай-ақ алмұрттың піспеген жемістерінің тілімдеріне сынақтар жүргізу барысында, бұл өнім олардың қоңырқай түске боялуына әкеп соқты. Жалпы, алмұрттың піспеген жемістерінің тілімдеріне жүргізілген сынықтарында әртүрлі рецептуралар мен бактериялық изоляттар арасындағы ең жоғары тиімділікті A15 (Pantoea agglomerans) бактериялық изолятымен өңдеу кезінде (96%) екені анықталынды; басқа да антибиотик негізінде тиімді препараттар Kasumin 2L (94%) және Miedzian 50WP (89%) болды. Айта кету керек, құрамында Cu бар Controlphyt Cu бақылау препараты, тамырдан тыс және топырақ тыңайтқышы, 0,4% концентрациясын енгізген кезде 71%-ға дейін және 0,2% кезінде 41%-ға дейін жоғары тиімділікті көрсетті.

Түйін сөздер: алюминий фосэтилі, мыс, касугамицин, биоконтроль, алма, алмұрт.

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Эффективность химических продуктов и эпифитных бактерий в борьбе с бактериальным ожогом (*Erwinia amylovora*)

Аннотация. В модельных испытаниях, проведенных в теплице на яблонях, было показано, что в условиях высокого инфекционного давления E. amylovora однократное профилактическое применение Aliette 80WG в период полного цветения эффективен в снижении бактериального ожога пределах 60-70%. Не было никакого значительного увеличения эффективности после использования этого продукта дважды по сравнению с однократным применением. Однако Aliette оказалась неэффективной в защите терминальных побегов. Высокая эффективность, как в защите цветков, так и побегов яблони, была показана препаратом на основе медного купороса Miedzian 50WP, но он показал фитотоксичность на лепестках цветов. Также в опыте на ломтиках незрелых плодах груш этот продукт вызвал значительное побурение. В целом, наибольшая эффективность среди различных тестируемых рецептур и бактериальных изолятов была зафиксирована при обработке ломтиков незрелых плодов груш бактериальным изолятом A15 (Pantoea agglomerans) (96%); другими эффективными препаратами были антибиотик на основе Kasumin 2L (94%) и Miedzian 50WP (89%). Стоит учитывать, что контрольный препарат Control-phyt Cu, внекорневое и почвенное удобрение, содержащее Cu, показал достаточно высокую эффективность – до 71% при внесении в концентрации 0,4% и 41% при 0,2%.

Ключевые слова: фосэтил алюминия, медь, касугамицин, биоконтроль, яблоко, груша.

Introduction

Fire blight (*Erwinia amylova*) belongs to the most harmful diseases of apple, pear and over 130 other plant species, mainly of the *Rosaceae* family (van der Zwet et al., 2012). It occurs in almost 50 countries of all over the world causing losses of economic importance (EPPO 2017). The pathogen infects all organs of the above ground part of plants but the most susceptible are blossoms and terminal shoots. The resulting necrosis and cankers often lead to the death of a part or entire plants. Occurrence of fire blight depends primarily on inoculum source, susceptibility of the hosts and the course of weather conditions. In some years its severity is very high but in others it is noticed only on the restricted area. The main source of fire blight dissemination, especially on longer distances, are latently infected nursery material (van der Zwet and Walter, 1996). The important vectors of *E. amylovora* are also insects and birds (Mazzucchi 1994, Beach-Andersen, 1974, van der Zwet et al., 2012).

On the area of the Republic of Kazakhstan fire blight was first discovered in the early 2000s and from this time it disseminated to main regions of apple and pear growing. Favorable weather conditions, particularly in the south-east part of the country, contributed to the development of the disease and great losses, especially in apple production (Жармухамедова Г.А. и др, 2016). According to current data given under the program for the development of the agro-industrial complex in the Republic of Kazakhstan for 2013-2020 «Агробизнес-2020», the contaminated area covers of 419.935 ha, including Almaty oblast – 688 ha, Zhambyl oblast – 107.3 ha and South Kazakhstan oblast – 53 ha (Program for the development of the agro-industrial complex in Kazakhstan, 2013). However, actually these values are much higher.

The plant protection program against fire blight consists skillful integration of various methods including chemical, agrotechnical and biological onces. It is directed to elimination of disease source, protection against infection and decreasing of plants susceptibility (Steiner 2000; Sobiczewski, 2011). However, assortment of chemical products recommended to control of fire blight is extremely narrow and mainly based on copper compounds which are quite effective, but act only protectively and do not cure infected plants. Those preparations can be also phytotoxic which is manifested by fruit and leave russeting. In some countries, fungicide based on fosetyl of aluminium and antibiotics are registered (Psallidas and Tsiantos 2000; Deckers et al. 2011; Schoofs et al, 2014; Лазарев А.М., 2014). The use of streptomycin, which is highly effective in control of fire blight, create a risk of resistance induction of *E. amylovora* and other bacteria, e.g. the human

and animal pathogens. Therefore in EU the adoption of antibiotics in plant protection is prohibited (Council Directive 2004/129/EC). Also in Russia, application of antibiotics for agricultural purposes is not recommended, but drugs such as Fitolavin, which consists of streptomycin-type antibiotics synthesized by *Streptomyces griseus*, are intended for control of plant diseases, including fire blight (Kruglyak E.B. et al., 2009). In the Republic of Belarusia 4 preparations (Baktogen, Ecogrin, Betaprotectin and Enatin) based on selected bacteria that strongly inhibit growth of the *E. amylovora* have been developed (Hudnitska E.S. et al., 2015). To increase the resistance of trees, it is worth considering using prohexadione calcium-based products (Regalis, Apogee) (Norelli et al. 2003; Sobiczewski and Buban 2004; McGrath et al. 2009; van der Zwet et al. 2012). Among the new perspectives are these of natural origin, including biopreparations and resistance inducers.

In recent years at Kazakh Research Institute for Plant Protection and Quarantine in Almaty the research on the biology and harmfulness of the causal agent of a fire blight is conducted. To control of the disease it is proposed various agrotechnical and chemical measures, among others Japanese antibiotic product Kasumin 2L, which is temporarily registered in Kazakhstan. However, Sagitov et al. (2016) believes that recommended foreign products for use in Kazakhstan's orchards are very expensive and not effective enough due to lack of the adaptation to specific ecological conditions (Vernigor A.et al., 2016). In this regard, at the Research and Production Center of Microbiology and Virology in Almaty has begun research on the development and creation of domestic means of protecting fruit crops based on microorganisms and biologically active substances against *E. amylovora*.

The aim of our study was to evaluate the efficacy of copper (Miedzian 50WP) and fosetyl aluminium (Aliette 80WG) products, Kasumin (kasugamycin) and 5 bacterial isolates originating from apple phyllosphere.

Material and methods

Efficacy of fungicides on apple blossoms

Apple blossoms on potted one-year old 'Idared' and 'Szampion' trees growing in the greenhouse were sprayed at full blooming once with water suspension of copper preparation Miedzian 50 WP (50% copper oxychloride) at a dose of 1.5 kg/ha or with Aliette 80 WG (80% of fosetyl aluminium) at a dose of 3.75 kg/ha, once or twice (the second treatment was done 3 days later). After 48 h treated blossoms were inoculated by spraying with a water suspension of the highly virulent strain Ea659 of *E. amylovora* (10^7cfu/ml) and immediately covered with plastic bags for 24 hours. On 5th and 7th day after inoculation, the presence of fire blight symptoms on the blossoms was recorded using a slightly modified scale of Pusey (1999): 0 – apparently healthy blossom, 1 – necrosis visible on sepals or petals and/or blossom bottom, 2 – necrosis in the whole ovary, 3 – necrosis additionally covering a half the length of the peduncle, 4 – total necrosis of ovary and peduncle (Fig. 1). Each treatment was tested on 160 blossoms (4 replicates x 40 blossoms on 5-10 trees).

Figure 1 – Efficacy of fungicides on apple blossoms

Efficacy of fungicides on terminal apple shoots

Tips of actively growing shoots on one-year-old apple trees cv. Idared growing in pots in the greenhouse were cut off with sterile scissors below first developed leaf and afterwards sprayed once with water suspension of Miedzian 50 WP at a dose of 1.5 kg/ha or Aliette 80 WG at a dose of 3.75 kg/ha, once or twice (the second treatment was done 3 days later).

Inoculation by spraying with strain Ea659 at concentration of 10^7cfu/ml was done 48 or 72 h after the last treatment. After inoculation the shoots were covered with plastic bags for 24 hours. On 5th, 9th and 15th day after inoculation the measurement of total length of shoots and the length of their necrotized part was made (Fig. 2). The diseased part of the shoots was expressed as the percentage of its total length (Sobiczewski et al. 2015). Each treatment was tested on 16 shoots (4 replicates x 4 shoots on 4 trees).

Figure 2 – Efficacy of fungicides on terminal apple shoots

Efficacy of fungicides and bacterial isolates on pear fruitlets

The study object was commercial products recommended for control of fire blight: Kasumin 2L (kasugamycin), Controlphyt Cu (copper gluconate) and Miedzian 50 WP (copper oxychloride) as well as 5 bacterial isolates originating from apple phyllosphere. The method described by Sobiczewski and Millikan (1985) was used. The pear fruitlet slides of cv. Conference, at a thickness of 7–8 mm, were momentarily dipped into water suspension of tested product or bacterial isolate (10^8cfu/ml) and then placed on moisture filter paper in Petri dishes. After 6 hours the slices were inoculated with *E. amylovora,* strain Ea 659 by spraying with its water suspension at 10^7cfu/ml. The symptoms occurrence on slices was evaluated after 3, 4, 5 and 7 days of incubation at room temperature. The following scale was used: 0 – no symptoms, 1 – several drops of ooze, 2 – about half of slice with ooze drops and necrosis, 3 – more than half of slice with necrosis and heavy ooze, 4 – ooze and necrosis of whole slice (Fig. 3). Each isolate was tested on 40 fruitlet slices (4 replicates x 10 slices).

Fungicide phytotoxicity on pear fruitlets

Pear fruitlet slices at a thickness of 7–8 mm of cv. Conference were momentarily dipped into an aqueous suspension of tested preparation and then placed in Petri dishes on moist filter paper in room temperature. After 3 days the phytotoxicity was evaluated using 4 – degree scale: 0 – lack of symptoms; 1– natural discoloration after injury of fruitlet; 2 – russeting of about half of slice; 3 – russeting of whole slice. Chemical preparations: Miedzian 50WP (copper oxychloride), Champion 50 WP (copper hydroxide), Miedzian Extra 360 SC (copper oxychloride), Nordox 75 WG (copper oxide) were evaluated.

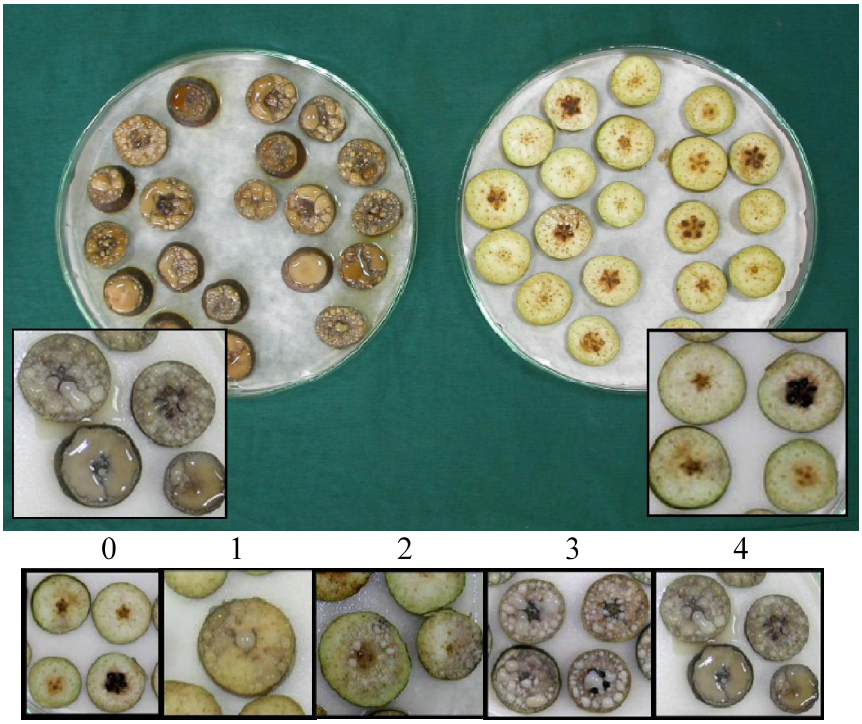


Figure 3 – Efficacy of fungicides and bacterial isolates on pear fruitlets

Statistical analyses

The results were elaborated using ANOVA on non-transformed data. For comparison of means, the Newman-Keuls test was used at the significance level of $\alpha = 0.05$ using STATISTICA 10.0 software.

Results and discussion

The study showed that protective treatment of apple blossoms with Aliette 80 WG and Miedzian 50 WP, applied once during full blooming, significantly reduced blossom blight (Table 1). Evaluation of disease severity on blossoms of cv. Szampion performed 5 days after inoculation showed 70% efficacy of the first of the mentioned products and the other – 100%. Slightly lower efficacy of both products was noted after the next two days of evaluation. Two-fold use of the Aliette deserves attention. The evaluation done both on 5th and 7th day after inoculation indicate a slightly smaller blossom infection, but insignificant in relation to the single use of this product, and thus a little higher efficacy. Generally, the similar efficacy of both products was found in the experiment on 'Idared' trees. In the case of Aliette, the severity of blossom blight was similar, regardless of the number of treatments.

Table 1 – Efficacy of chemical products in protection of apple blossoms against fire blight

Treatment	Disease severity in days after inoculation		
	cv. Szampion		cv. Idared
	5	7	5
Control (water)	0.70 c*	2.01 c	0.54 b
Aliette 80 WG 3.75 kg/ha A	0.21 b [70.0]	0.79 b [60.7]	0.13 a [75.9]
Aliette 80 WG 3.75 kg/ha BA	0.15 b [78.5]	0.68 b [66.2]	0.16 a [70.4]
Miedzian 50 WP 1.5 kg/ha A	0.0 a [100.0]	0.03 a [98.5]	0.0 a [100]

* degree of infection 0-4;

Application A – 90% of flowers open; BA – 10% and 90% of flowers open; statistical analysis was performed separately for each term; means with the same letters are not significantly different at $P<0.05$ according to Newman-Keuls test, data in brackets show the efficacy in % (degree of blossom infection treated with suspensions of the bacteria or commercial product in relation to control).

On apple shoots cv. Idared, the protective activity of Aliette was significantly different from that of Miedzian at all assessment dates, i.e. 5, 9 and 15 days after inoculation (Table 2). After a single application of both products the efficacy of Miedzian 50WP was 100, 92.2 and 64.8%, while that of Aliette – 41.8, 27.5 and 20.1%, respectively. In the first two evaluation dates, the severity of the fire blight on the shoots after treatment with Aliette did not differ significantly, regardless of whether one or two treatments had been applied. However, the assessment made after 15 days from the inoculation of shoots Aliette showed even a lack of efficacy of this product after two applications. In the second experiment, the assessments performed 5 and 9 days after inoculation also showed a lack of efficacy of Aliette. Miedzian, according to the assessment at 5 days after inoculation showed 100% efficacy but after another 4 days it decreased to 42.6%. Next assessment made after 15 days showed a lack of efficacy of Aliette when applied once, and significantly higher, although unsatisfactory, after two applications of this product. Whereas the most effective proved to be again Miedzian, but its efficacy was only 37.6%. Evaluation of the phytoxicity of Aliette on pear fruitlet slices showed that it was significantly less toxic than the standard copper oxychloride and oxide products (Fig. 4).

Table 2 – Efficacy of chemical products in protection of apple terminal shoots cv. Idared/M26 against fire blight

Treatment	Disease severity in days after inoculation					
	Experiment 1			Experiment 2		
	5	9	15	5	9	15
Control (water)	18.2 c	44.8 b	72.2 c	20.6 b	52.8 b	63.8 c
Aliette 80 WG 3.75 kg/ha **A**	10.6 b [41.8]*	32.5 b [27.5]	57.7 b [20.1]	17.0 b [17.5]*	46.8 b [11.4]	58.8 c [7.8]
Aliette 80 WG 3.75 kg/ha **BA**	7.9 b [56.6]	34.5 b [23.0]	71.6 c [0]	19.7 b [0]	44.3 b [16.1]	49.1 b [23.0]
Miedzian 50 WP 1.5 kg/ha **A**	0.0 a [100]	3.5 a [92.2]	25.4 a [64.8]	0.0 a [100]	30.3 a [42.6]	39.8 a [37.6]

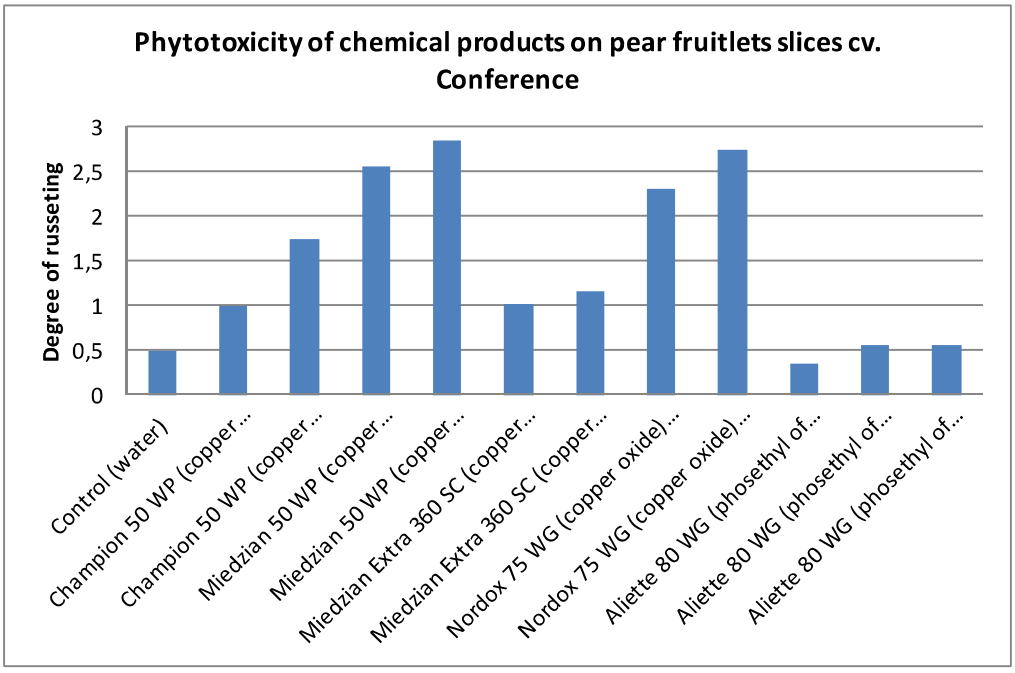
BA – 3 days interval between treatments.

Inoculation was done 72 h after last Aliette 80 WG treatment and 6 h after Miedzian 50 WP treatment.

'1' percentage of shoots infestation: the length of the shoot's necrosis in relation to its total length; data in brackets show the efficacy in % (percentage of shoots infestation treated with the respective bacteria in comparison to control); statistical analysis was performed separately for each term; means with the same letters are not significantly different at $P<0.05$ according to Newman-Keuls test (means ± SE) (means ± SE).

Aliette is an acidic systemic fungicide used primarly against plant diseases caused by *Phythophthora* spp. and some species of fungi. However, it also has been found to be effective against certain plant bacterioses. The results of experiments conducted in both the USA and Europe showed that efficacy of Aliette in control of fire blight was inconsistent (Psallidas and Tsiantos 2000). Norelli and Aldwinckle (1993) reported that the use of this product gave poor efficacy in protection of apple blossoms under both high or low inoculum pressure. In tests of Clarke et al. (1993) the treatments with Aliette provided a significant reduction in the number of naturally occurring blighted blossom clusters under highly favorable conditions for infection. However, no differences between treated and untreated trees were evident under less favorable conditions. Aliette was also not efficient in protection of apple terminal shoots. Tsiantos and Psallidas (1993b, 1996) found no significant effect of fosetyl-Al in either preventive or curative sprays with artificial inoculations, while with natural infections a significant effect of Aliette, probably because of low inoculum pressure, was observed (Tsiantos and Psallidas, 1996a). In contrast, Burr and Norelli (1984) showed effective control of fire blight with fosetyl-Al, equal to that of streptomycin. No significant difference in dose effect was found, probably because of unfavorable climatic conditions for disease development. Paulin et al. (1990) reported that in the orchard conditions sprays with relatively high dosage on blossoms of pear or apple were often efficient in reducing the number of infection. However, results were

inconsistent. No curative effect had been noticed. Another trials conducted over four years showed that application of fosetyl-Al before expected infection during bloom was effective against disease on apple and pear trees (Laurue and Gaulliard 1993). Hagan and Akridge (2002), however, proved on three-year studies with various bactericides performed on field-grown crabapple trees, that no significant limitation of fire blight severity was obtained in case of Aliette application. On the other hand Deckers et al. (2011, 2013) documented a substantial reduction of bacterial ooze formation in infected area of apple and pear blossoms, shoots and fruits when the trees were preventively treated with fosetyl-Al. This indicate the possibility of inoculum source reduction in the orchard.



*Mean values with the same letter do not differ significantly according to the Newman-Keuls test

Figure 4 – Phytotoxicity of chemical products on pear fruitlets slices cv. Conference

Copper compounds have been established as effective bactericides and have been used against fire blight on apples and pears since 1900 (van der Zwet et al., 2012). First commercial product based on copper was Bordeaux mixture. In next years the products containing copper hydroxide, copper oxychloride and cuprous oxide as active ingredients were developed. All copper formulations, with artificial or natural infections, were equally or more effective against fire blight and some were comparable with streptomycin. However, all these products are phytotoxic at the doses recommended for efficient fire blight control during bloom and post-bloom (Psallidas and Tsiantos 2000).

Our evaluation of efficacy of Kasumin 2L, Controlphyt Cu and 5 bacterial isolates on pear fruitlets showed that the best protective activity demonstrated isolate A15, identified as *Pantoea agglomerans* (Table 3). The severity of fire blight on treated with this isolate fruitlets, evaluated 7 days after inoculation, was lowest out of all products and bacteria tested. Similar effect gave Kasumin and included for comparison Miedzian at both concentrations. Noteworthy is product Controlphyt Cu, the soil and foliar fertilizer, containing only 6.5% Cu, complexed with gluconic acid soluble in water. Any phytotoxic effect was observed after its application on pear fruitlet slices (data not shown). Efficacy of 71% and 42% determined a week after inoculation after application at concentrations of 0.4 or 0.2% respectively, under high *E. amylovora* infection pressure should be considered satisfactory. Antibiotic based product Kasumin is now proposed for replacement use with streptomycin where resistance to it was detected. Its active ingredient kasugamycin belongs to the same

class as streptomycin but differs in mode of action (Copping and Duke, 2007). Study of McGhee and Sundin (2011) showed that Kasumin used against apple blossom blight was statistically equivalent to the industry standard streptomycin in all 6 experiments. Moreover in replicated lab experiments the development of spontaneous resistance in *E. amylovora* to 250 or 500ppm of kasugamycin was not observed when cell were directly plated on medium containing high concentrations of antibiotic. Considering all available products and means for fire blight control in Belgian conditions Shoofs et al. (2014) recommend a strategy including application of copper at the beginning of the season, Vacciplant (laminarin) and/or biopreparation Blossom Protect (*Aurebasidium pullulans*) during bloom and 3 times with Aliette at intervals of 10-14 days after petal fall. At the end of the season copper treatment can be made during leaf fall period to protect leaf scars.

Table 3 – Efficacy of bacterial isolates and commercial products in control of fire blight on pear fruitlets slices of cv. Conference.

Treatment	Severity of fire blight in days after inoculation			
	3	4	5	7
Control	1.00 c	2.00 d	3.00 f	3.91 d
A11*	0.90 c	2.00 d	2.00 e	3.62 d (7.0)
A12*	0.25 b	1.81 d	2.05 e	3.57 d (9.0)
A13*	0.00 a	0.60 bc	1.51 d	2.30 c (41.0)
A14*	0.30 b	1.88 d	1.88 e	3.72 d (5.0)
A15**	0.05 a	0.05 a	0.10 a	0.15 a (96.0)
Kasumin 2L 0,5%	0.00 a	0.07 a	0.12 a	0.25 a (94.0)
Controlphyt Cu 0,2%	0.12 a	0.83 c	1.22 c	2.27 c (42.0)
Controlphyt 0,4%	0.02 a	0.52 b	0.62 b	1.12 b (71.0)
Miedzian 50 WP 0,1%	0.02 a	0.10 a	0.20 a	0.45 a (89.0)
Miedzian 50 WP 0.3%	0.00 a	0.00 a	0.00 a	0.42 a (89.0)

* Gram negative bacterial isolates; ** *Panoea agglomerans;* Mean values with the same letter in columns do not differ significantly according to the Newman-Keuls test; values in brackets – efficacy

In summary, it should be emphasized that the main element of integrated management of fire blight is inspection of the *E. amylovora* host plants. The inspections should start at the end of blossom period or just after petal fall and be continue throughout the entire season. The use of all appropriate methods for management should be based on careful consideration directed toward suppressing damages to the acceptable threshold. Reducing primary infection source in the orchard and in its vicinity by removing holdover cankers during winter pruning and, if necessary, also during the vegetation period is extremely important. Early elimination of fire blight foci results not only reduction of potential loses but also in limitation of disease dissemination. This also includes application of copper sprays at silver- or green-tip stag. Some perspective creates growth retardant known in Europe as Regalis 10 WG and as Apogee in the USA. However, this product should not be recommended for young orchards. Integrated disease management includes choosing orchard sites and cultivars as well as fertilization, irrigation and cultivation practices. The relative susceptibilities of potential rootstock and cultivar should be given high priority.

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