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EFFECTS OF HARPIN PROTEIN AND HUMIC ACID ON SHOOT GROWTH AND FIRE BLIGHT DISEASE (*Erwinia amylovora* (Burr.) Winslow et al.) ON PEARS

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ABSTRACT

Harpin protein is critical to the virulence of Erwinia amylovora in host plants. Humic acid is reported to improve plant growth and resistance to plant diseases. In vitro and in vivo conditions, effectiveness of the systemic acquired resistance (SAR) inducer harpin protein, humic acid as a fertilizer, and bactericides copper and streptomycin were evaluated on pear cultivars, on shoot blight phase of the disease. Harpin protein was applied at the rate of 50 mg.L^{-1} at two shoot lengths of 15-20 cm and 30-35 cm, humic acid (200 mg.L^{-1}) was applied three times when the shoot lengths 6-12 cm, 15-20 cm and 30-35 cm. On cv. Ankara, harpin protein showed about 55% effectiveness, alone and the addition of copper had been the most effective treatments in both years, followed by streptomycin. In addition, it reduced the shoot blight phase of the disease on the inoculated seedlings and trees significantly compared to copper applications and untreated controls. None of the chemicals affected shoot lengths of plants statistically. Humic acid applications gave worse results within all of the chemicals in controlling fire blight on pear cultivars. In the bioassay test, on the contrary of humic acid, harpin protein reduced bacterial populations compairing to control plants in the leaves. Harpin protein should be seen as a complementary action in the whole process of fire blight control measures.

Key Words; *Erwinia amylovora, harpin protein, humic acid, pear, SAR*

HARPİN PROTEİN VE HUMİK ASİDİN ARMUTLARDA SÜRGÜN GELİŞİMİ VE ATEŞ YANIKLIĞI HASTALIĞI (*Erwinia amylovora* (Burr.) Winslow et al.) ÜZERİNE ETKİLERİ

ÖZET

Harpin protein, konukçu bitkilerde Erwinia amylovora' nın virulensi için kritik önemlidir. Humik asidin bitki gelişimini iyileştirdiği ve bitki hastalıklarına karşı dayanıklılık sağladığı rapor edilmektedir. In vitro ve in vivo koşullarda, sistemik kazanılmış dayanıklılığı (SAR) teşvik eden harpin protein, gübre olarak humik asit ve bakterisitler bakır ve streptomisinin, armutlarda hastalığın sürgün yanıklığı dönemindeki etkililikleri değerlendirilmiştir. Harpin protein, sürgün uzunlukları 15-20 cm ve 30-35 cm iken iki kez 50 mg.L^{-1}oranında, humik asit sürgün uzunlukları 6-12 cm, 15-20 cm ve 30-35 cm iken üç kez ve 200 mg.L^{-1} oranında uygulanmıştır. Harpin protein, Ankara çeşidinde %55 civarında etkililik göstermiş, tek başına ve bakırla birlikte her iki yılda, streptomisinden sonra en etkili uygulama olmuştur. Ayrıca harpin, fidan ve ağaçlarda hastalığın sürgün yanıklığını, bakır ve kontrole kıyasla önemli ölçüde azaltmıştır. Kimyasalların hiçbiri istatistiksel olarak sürgün uzunluklarını etkilememiştir. Humik asit uygulamaları, armut çeşitlerindeki hastalığın mücadelesinde tüm kimyasallar içinde en kötü sonuçları vermiştir. Biyoassay testlerinde, humik asidin tersine, harpin protein yapraklarda kontrol bitkilere kıyasla bakteriyel populasyonları azaltmıştır. Harpin protein, ateş yanıklığının mücadele programı içersinde tamamlayıcı bir etki olarak görülmelidir.

Anahtar Kelimeler; *Erwinia amylovora, harpin protein, humik asit, armut, SAR*

INTRODUCTION

Erwinia amylovora (Burr.) Winslow et al. is the casual agent of fire blight, a destructive bacterial disease that affects principally pear and apple, and other rosaceous plants of economic importance, including other fruit trees and ornamentals (van der Zwet and Keil 1979). The most important chemicals for controlling fire blight disease in pome fruit trees are copper compounds and antibiotics. However, copper treatments often results russeting of fruits and antibiotics constitute potential risks of promoting the development of antibiotic resistance in bacterial pathogens. Due to the lack of effective, and non-phytotoxic preparations to combat fire blight, there has been much interest in recent times in novel control strategies.

Plant defense response has been shown to culminate in a number of physical and biochemical changes to systemic expression of defense proteins, causing 'systemic acquired resistance (SAR)', in the host plant designed to limit pathogen penetration and development in the host tissues (Dixon et al. 1994; Wei and Beer 1996; Agrios 1997; Momol et al. 1999; Anonymous 2000b).

Harpin protein (Messenger) which has been isolated from *E. amylovora* initiates a complex set of metabolic responses in the treated plant, causing natural gene expression and eliciting a plant's natural defence and growth systems. Harpin is an acidic, heat-stable, glycine-rich, extracellular protein with a molecular weight of about 40 kilodaltons. The protein consists of 403 amino acid residues with no cysteine (Anonymous, 2000a).

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Harpin protein activates natural growth systems, improving crop yield, quality and food safety while simultaneously triggering defense systems to protect against fungal, bacterial and viral diseases and pest damage but it exhibits no direct inhibitory or toxic effect on plant pathogens, and thus can not exert the selection pressure that would promote the development of resistance for pathogens and pests. The harpin protein firstly binds to plant's receptors. Plants are naturally equipped with early warning receptors that detect harpin proteins. Secondly, the receptors react to harpin as if it were a pathogen-stimulating the plant to act. The plant responds by sending a signal (or message) throughout itself, initiating a sequence of physiological and biochemical reactions. Thirdly, the plant reaction activates both growth and stress-defense pathways within the plant. The growth response is most pronounced, amplifying the plant's current processes. This response increases nutrient uptake, photosynthesis, vigor and reproductive activity of the plant. The stress-defense reponse improves plant stamina, increasing stress tolerance. Plant stress can be caused by environmental events, physiological shifts in plant growth and outside biological agents. The benefit of stress-defense response depends on the severity and duration of a particular stress condition. Finally, growth and stress-defense responses interact and contribute to overall plant health. Improved plant health can result in one or more of the following outcomes: increased yield, improved quality and / or extended shelf life (Wei et al., 1992; Beer et al., 1993; Kessman et al., 1994 and 1996; Sticher et al., 1997; Anonymous, 2000 a, b and c; Grisham, 2000; Aldwinckle et al., 2002; Anonymous, 2002; Fontanilla, 2005).

Humic acid is reported to improve plant vigor and natural resistance to plant diseases by the manufacturers. It helps to increase the yield about 70% and to decrease fertilizer and pesticide use approximately 30% (Freeman, 1969; Anonymous, 2000 d and e). Humic acids have the ability specifically to influence microbial metabolism of proteins and carbohydrates by catalytic means. This leads to a direct devastating effect against bacteria or viruses. A second mechanism is related to the inter-ionic bonds of high-molecular protein fractions (toxins) of infectious microbes. Their toxic impact on physiological processes of mucous membrane cells can be weakened considerably or even blocked completely.

Uptake of major plant nutrients is mediated by humic substances. Stimulative effect of humic substances on plant growth is enhanced uptake of major plant nutrients: nitrogen, phosphorus, and potassium. Researchers have reported increased uptake of calcium and magnesium when plants are applicated with liquid suspensions of humic acids. After applications of humic substances are applied changes in many different metabolic processes are detected. Enhanced carbohydrate production can either result in improved product quality or increased yields.

Foliar applications can be timed to activate vegetative growth, flowering, fruit set, or filling and ripening of fruits. Energy metabolism is accelerated and the chlorophyll content of plant leaves is enhanced by the presence of humic substances. As the chlorophyll concentration increases there is a correlated increase in the uptake of oxygen. During these metabolic changes an increase in the concentration of several important enzymes is detected. Some of the enzymes which are reported to increase are catalase, peroxidases, diphenoloxidase, polyphenoloxidases, and invertase. Some molecular components of humic substances act to regulate plant growth hormones. Both humic acids inhibit the enzyme, indole acetic oxidase thereby hindering IAA destruction (Senn and Kingman, 1973; Aiken et al., 1985; Mac Carthy et al., 1990; Senesi and Miano, 1994; Gaffney et al., 1996; Hayes and Wilson, 1997; Davies and Ghabbour, 1998).

The objective of this work is to determine the effectiveness of harpin protein as a plant activator and a fertilizer, humic acid, and their combinations with copper, consequently to get comparable efficacy with bactericides, copper and streptomycin, for shoot blight phase of fire blight disease on pear varieties.

MATERIALS AND METHODS

Plant material and growth conditions

The pear cultivars, Santa Maria, Williams, Ankara, Deveci and Riza Bey which are grown extensively, were used in the experiments. In greenhouse experiments, the test plants were selected among 3 year-old saplings and in field experiments, the trees of 11 years old showing uniform growth. These saplings were transplanted into plastic pots of 25 cm diameter filled with 8 kg of soil and they were grown at 25 ± 5 ^{0}C, 60–75 % RH and under 12000–14000 Lux from tungsten-filament lamps for a 16-h photoperiod for 20 days. After transplantation, the trees were fertilized once a week with 25g/pot ammonium sulfate, 25g/pot diammonium phosphate, 25g/pot potassium sulfate, and 50 ml/pot of a liquid fertilizer having 0.05% Mn, Cu, Zn, B, Mo (Kacar and Katkat, 1999). In addition, sulfur dust was applied once (4g/L water) for powdery mildew control. In the beginning of growing season, pear trees were prunned, fertilized and sprayed to prevent insect injury for healty growth of plants in field.

Erwinia amylovora strain

After conducting virulence tests on cv. 'Ankara' pear trees, a virulent strain of *E. amylovora* (EAI), was selected for all inoculations (Norelli et al., 1984). Stock cultures were preserved at 4 ^{0}C on the nutrient agar (NA) medium and transferred to new tubes every three months.

Bacterial suspensions prepared from growing colonies on NA at 23–25 °C and were diluted in sterile distilled water (SDW) to give an absorbance of 0.15 at

660 nm. This represented 10^8 cfu ml^{-1} based on viable plate counts. Inoculum was maintained on ice and was used for plant inoculation within 2 h of dilution.

Chemical compounds used in the experiments and their applications

The chemical compounds used in the experiments are: harpin protein, humic acid, copper salts and streptomycin. These compounds and their properties are shown in Table 1. Chemical application timing and schedule were based on Momol et al. (1999) (Table 2). Harpin protein was applied twice when the shoot lengths were 15-20 cm and 30-35 cm, copper salts and humic acid were applied three times when the shoot lengths were 6-12 cm, 15-20 cm and 30-35 cm and streptomycin was applied twice, one day before and one day after the inoculation (Momol et al., 1999). Streptomycin and copper treatments were employed as positive controls.

Table 1. Active ingredients, application rates, formulations of chemical compounds used in the experiments

Active Ingradient and Percentage	**Commercial Name / Firm**	**Formulation**	**Application Dose (100 L water)**
Harpin protein %3	Messenger® / Eden Biosci.	Powder	50 g***** +20 ml adjuvant******
Humic acid %55 Fulvic acid %30 Potassium hydroxide %8	K-humate / Hektas Comp.	Granule	200 g
Streptomycin sulfate %100	Streptomycine / I.E. Ulagay	Powder	59 g
Copper salts of fatty and rosin acids %51.4	Tenn Cop 5E / Hektas Comp.	Liquid	250 ml

** Prepared in distilled water*

***Non ionic adjuvant, KINETIC® was manufactured for Helena Chemical Company*

Table 2. Chemical Treatments, date of chemical application and date of inoculation with *E. amylovora* to pear plants

Treatments	**Application times and Shoot lengths (cm)**					
	May[4] (6-12cm)	June[5] (15-20cm)	June[6] (30-35cm)	July[7] (40-45cm)	July* (40-45cm)	July[8] (40-45cm)
Harpin protein[1]		x	x		x	
Harpin protein+Copper[2]		x	x		x	
Harpin protein[3]		x	x			
Humic acid[1]	x	x	x		x	
Humic acid+Copper[2]	x	x	x		x	
Humic acid[3]	x	x	x			
Copper[1]	x	x	x		x	
Copper[3]	x	x	x			
Streptomycin[1]				X	x	x
Streptomycin[3]				X		x
Control (water)[1]	x	x	x		x	
Control (water)[3]	x	x	x			

**Inoculation with Erwinia amylovora after application of chemical, on June 26th , 2002 and June 19th, 2003*

[1] Chemical + E. amylovora inoculation

[2] Chemical + Copper + E. amylovora inoculation

[3] Only chemical / water for control plants

[4]Treatments applied May 31st, 2002 and May 24th, 2003

[5]Treatments applied June 10th, 2002 and June 3th, 2003

[6]Treatments applied June 20th, 2002 and June 13th, 2003

[7]Treatments applied July 25th, 2002 and July 18th, 2003

[8]Treatments applied July 27th, 2002 and July 20th, 2003

Experimental design and setup

The experiment was set up in a completely randomized block design with 3 replicates. A single replicate was a mean from nine shoots on three saplings (Duzgunes et al., 1987). Each treatment was applied to five groups of plants (Table 3). The first three group of plants being treated by the chemicals and inoculated with *E. amylovora* to see the effects of chemicals on the disease severity (first group treated with chemicals + *E. amylovora* inoculation, second group treated with chemicals + copper compound + *E. amylovora* inoculation, third group as control 1 treated only with *E. amylovora* inoculation). The fourth group was treated only with the chemicals, and the fifth group treated with water as control 2 to see

the effects of treatments on shoot growth of loquat and quinces. The different combinations of treatments were all analyzed as separate treatments. The experiments were conducted in two growing seasons, 2002 and 2003.

Table 3. Experimental design for applications on pear cultivars

Plant Groups	*Applications*
First group plants	*Chemical + *E. amylovora* inoculation
Second group plants	Chemical (except Copper and Streptomycin) + Copper + *E. amylovora* inoculation
Third group plants (Control$_1$= for disease severity)	*E. amylovora* inoculation
Fourth group plants	Chemicals
Fifth group plants (Control$_2$= for shoot growth)	Water

**harpin protein, humic acid, copper compound, streptomycin*

Inoculation of the shoots

Actively growing shoot tips of plants were inoculated by inserting a 0.46-mm-diameter (26-gauge) hypodermic needle through the stem just above the youngest unfolded leaf. A suspension of 10^8 cfu/ml *E. amylovora* was introduced to fill the wound and leave visible drops at both ends of the wound. The treated shoots were labeled with flagging tape for evaluation purposes (Norelli et al., 1986).

Evaluation of disease severity and shoot growth

The lengths of visible fire blight lesions and of the current season's shoot growth were recorded after all lesions had ceased to extend, as determined by the formation of a determinate margin between diseased and healthy tissue. Disease severity was calculated by the following formula: Disease severity (%) = (a / b) x 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). Percent effectiveness of the applications (**A**) was calculated according to the following formula of A = 100 x (B – C) / B; where **B** is the percent disease severity in the controls, **C** is percent disease severity in treated shoots. Percent effectiveness of the treatments on reduction of shoot growth (**D**) was calculated in a similar way, D = 100 x (E – F) / E; where **E** is the mean shoot length in the controls, and **F** is the length of treated shoots (Anonymous 1996).

MINITAB (State College, PA, USA) was used to determine statistical program. The means (expressed as percent disease) were used to determine significant treatment differences in the MINITAB statistical program. Data was analyzed using MSTAT software (Michigan State University, USA) and the differences between factors the treatments were determined by Duncan's New Multiple Range Test.

Determination of the bacterial population in plant tissues

Simultaneously the bacterial development in the plant tissue was determined. Leaves from shoot tips were taken from each plant on the 5th, 10th and 15th days after the treatments and inoculation by the pathogen. 1 g leaf was homogenized in 10 ml phosphate buffered saline (PBS: 10 mM, pH 7.2; NaCl 8 g; KCl 0.2 g; $Na_2HPO_4 \cdot 12H_2O$ 2.9 g; KH_2PO_4 0.2 g; distilled water to 1 L.) in a mortar and each homogenate diluted 1-6 times. From each homogenate a dilution plating was made on 5% Saccarose Nutrient Agar (SNA) and incubated for 2-3 days at 27 ^{0}C (Lelliott and Stead 1987). Amount of bacterial population in plant tissue was calculated according to Klement et al. (1990) with the following formula; Bacterial Population in Plant Tissues = *Number of colonies x Dilution of sample x 10*

RESULTS

Effectiveness of the chemicals on disease severity

Effects of harpin protein, humic acid, and their combinations with copper compound, alone copper compound and streptomycin were determined based on disease severity in comparison to the untreated control on pear cultivars. Statistically, there were interactions between pear cultivars, chemicals and years ($p< 0.01$) both in 2002 and 2003. In all of the pear cultivars, harpin protein and combination of its with copper compound controlled the shoot blight phase of fire blight as hopeful and successful chemical following streptomycin treatments in two years. The effectiveness of harpin protein on fire blight severity, on cv. Ankara, 35.13% and 42.41% in the greenhouse, 50.23% and 55.83 in the field, was far greater ($p< 0.01$) than that of bactericide copper compound, which resulted reduction, 6.57% and 9.37% in the greenhouse, 6.51% and 12.33% in the field in 2002 and 2003, respectively. Addition of copper did not increase the effectiveness of harpin protein significantly. Copper compound alone was not effective at the expected level. Humic acid applications gave identical or worse results than untreated controls on fire blight disease severity in the greenhouse and field experiments. Considering datas, it was determined disease severities with humic acid treatments on cv. Ankara as 84.51 and 80.76 in greenhouse and 85.06 and 72.79 in field in 2002 and 2003, respectively.

According to obtaining disease severity results in controls, cv. Ankara showed the lowest disease severity in both years and greenhouse and field conditions ($p<0.01$) (Table 4 and Table 5).

Effectiveness of the chemicals on shoot growth

Effects of the applied chemicals on shoot growth yielded numerically some differences in comparison to the untreated control on pear cultivars, but the difference was not statistically significant. Contrary to expectations, identical results with untreated control were obtained on saplings and trees with humic acid applications (Table 6).

Determination of the bacterial population in plant tissues

Taking on the leaves from shoot tips of each plant which treated by chemicals and inoculated by *E. amylovora* on the 5th, 10th and 15th days, simultaneously the bacterial population was determined according to Klement et al. (1990) in the plant tissue. Statistically, there were interactions between varieties, chemicals and days ($p<0.01$) and differences between the treatments, determined by Duncan's Multiple Range Test.

According to our findings, the lowest bacterial populations ($0,3x10^3$, $0,7x10^4$ and $0,8x10^3$ on cv. Ankara) were obtained by streptomycin on all of the varieties and in all days and it was followed by harpin protein on pear cv. Ankara with $0,9x10^3$, $1,2x10^5$ and $1,1x10^4$ on the 5th, 10th and 15th days, respectively. In addition, harpin protein applications gave lower bacterial density compare to copper compound; $4,4x10^4$, $4,3x10^7$ and $2,9x10^5$ and control; $4,4x10^5$, $5,0x10^7$ and $4,4x10^5$ on cv. Ankara on the 5th, 10th and 15th days, and this was followed by cv. Williams, cv. Santa Maria, cv. Riza Bey and cv. Deveci, respectively. Results of humic acid applications were almost the same as the controls (Figure 1).

DISCUSSION

The shoot blight phase of fire blight caused by *E. amylovora* is highly destructive within the current and subsequent growing seasons and improved strategies are required for the control of fire blight on pome fruits. Danovan (1991) and Beyers and Yoder (1997) reported that the first factor determining the susceptibility of the host plant against shoot infections of fire blight was rapid shoot growth.

Harpin protein provided broad spectrum protection of plants against fungal, bacterial and viral pathogens (Wei and Beer 1996; Momol et al. 1999; Jones, 2001; Anonymous, 2002; Fontanilla et al. 2005). Since harpin is clearly required by *E. amylovora* for pathogenicity, interference with harpin or its activity may provide new bases for the control fire blight (Beer et al. 1993). According to data obtained from our experiments, in all of the pear cultivars, harpin protein generally was provided better shoot blight control compared the control plants following by streptomycin. As similar, Günen et al. (2006), harpin were applied to 10-year-old pear trees of the fire blight susceptible cv. Dr. Jules Guyot and results of the trials were streptomycin sulfate; 17.53%, Harpin; 35.04%, control; 65.23% in 2002, streptomycin sulfate; 15.80%, Harpin; 33.39%, and control; 66.97% in 2003.

From the point of view of susceptibility of pear varieties and disease severities, obtained data from greenhouse and field applications gave similar results and supported by bioassay test about bacterial populations in leaf tissues. Even if it was found successful compared to copper compound, obtaining low disease control by harpin protein can be attributed to the inoculation method, high inoculum density, and cultivar susceptibility. If these situations were taken into consideration, better results might be obtained in the natural infections. So repeated applications in current season should be considered in situations where disease epidemics are anticipated.

Streptomycin was effective at preventing the shoot blight phase of the disease on pears, however, the use of this chemical must be limited to high disease pressure conditions.

In the control of fire blight, copper compounds can be effective only at low and medium disease severities (van der Zwet and Keil, 1979) and the rate of control is lower on susceptible host pears (Dimova, 1990). We obtained very low disease control of shoot blight phase of fire blight from copper compounds alone or in combination with harpin protein and humic acid on pears. Romero et al. (2001) also found that addition of copper compounds to plant activators did not affect the performance of the plant activators. In contrast to our data from this study, some researchers obtained increasing yield and lower disease onset by the application of plant activator +fungicide mixtures (Anonymous 1997). Addition of copper salts of fatty and rosin acids did not improve but reduced the effectiveness of some of the chemicals.

Humic acid applications were ineffective as a fire blight disease control both on pear cultivars. Humic acid should not be used as foliar application on pears in the growing season. This negative effect should be further tested under different climatic conditions and with different application doses. As an interesting result, humic acid applications did not effect shoot growth statistically, although it was used in early stages of plant and in the contrary of expecting.

It is important to note that host resistance inducers have to be applied prophylactically against pathogen infections; they should be used 1-3 weeks prior to a possible infection risk or inoculation by *Erwinia amylovora*. It will be necessary to find the right strategy for the applications of these compounds in different areas. Harpin protein should be seen as a complementary action in the whole process of fire blight control measures.

Table 4. Effectiveness of the chemicals on disease severity caused by *Erwinia amylovora* on pear cultivars in greenhouse conditions in 2002 and 2003

Chemicals	cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey	
	[1]D.S.* (%)	[2]E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)
2002										
Hrp	**52.82 uvw	35.13	64.51 qr	28.63	62.19 qrst	33.52	63.69 qrs	31.10	74.14 nop	19.72
Hrp+Copper	50.67 vwx	37.77	66.59 pq	26.33	61.77 qrstu	33.97	74.45 nop	19.50	72.84 op	21.13
Humic acid	84.51 abcdefghijkl	0.00	88.48 abcdefgh	2.12	86.02 abcdefghij	8.06	89.47 abcdefg	3.26	92.05 abcd	0.33
HA+Copper	80.29 ghijklmno	1.39	86.69 abcdefghi	4.10	82.67 defghijklmn	11.63	87.62 abcdefgh	5.26	93.80 a	0.00
Copper	76.08 klmno	6.57	79.07 hijklmno	12.53	81.84 efghijklmno	12.52	85.60 abcdefghij	7.44	84.69 acdefghijk	8.30
Streptomycin	3.50 z	95.70	4.87 z	94.61	5.23 z	94.41	4.44 z	95.19	28.77 y	68.85
Control	81.43 fghijklmno	0.00	90.40 abcdef	0.00	93.56 ab	0.00	92.49 abc	0.00	92.36 abc	0.00
2003										
Hrp	47.67 wx	42.41	57.91 qrstuv	33.46	54.45 tuvw	35.22	55.77 rstuvw	35.50	53.98 tuvw	34.97
Hrp+Copper	44.07 x	46.76	58.19 qrstuv	33.14	56.48 rstuvw	32.80	54.07 tuvw	37.46	54.87 stuvw	33.90
Humic acid	80.76 ghijklmno	2.44	91.08 abcde	0.00	85.12 abcdefghijk	0.00	92.07 abcd	0.00	87.75 abcdefgh	0.00
HA+Copper	79.62 hijklmno	3.81	84.99 abcdefghijk	2.35	81.09 fghijklmno	3.53	85.70 abcdefghij	0.89	80.10 ghijklmno	3.51
Copper	75.02 mnop	9.37	77.22 ijklmno	11.28	76.53 jklmno	8.95	80.65 ghijklmno	6.73	75.16 lmnop	9.46
Streptomycin	5.86 z	92.92	3.03 z	96.51	2.60 z	96.90	2.03 z	97.65	4.16 z	94.98
Control	82.78 defghijklmn	0.00	87.04 abcdefgh	0.00	84.06 bcdefghijklm	0.00	86.47 abcdefghi	0.00	83.02 cdefghijklmn	0.00

[1]D. S.; Disease Severity, [2]E. C.; Effectiveness of Chemical, *There was an interaction statistically between *disease severities* and *chemicals* and *years* in two years, figures are averages of three replications, each consisting of three shoots ($P<0.01$)

Table 5. Effectiveness of the chemicals on disease severity caused by *Erwinia amylovora* on pear cultivars in field conditions in 2002 and 2003

Chemicals	cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey	
	[1]D.S.* (%)	[2]E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)	D.S. (%)	E.C. (%)
2002										
Hrp	**37.25 wx	50.23	64.94 rstuv	23.25	62.77 v	26.24	66.23 qrstuv	24.98	68.99 opqrstuv	15.18
Hrp+Copper	40.06 w	46.47	63.70 tuv	24.72	64.42 stuv	24.30	71.39 klmnopqr	19.14	69.65 nopqrstu	14.37
Humic acid	85.06 abc	0.00	80.41 bcdefgh	4.97	82.92 abcde	2.57	85.77 ab	2.85	84.53 abcd	0.00
HA+Copper	79.96 bcdefgh	0.00	80.02 bcdefgh	5.43	77.91 defghijk	8.45	81.71 bcdef	7.45	79.28 bcdefghi	2.53
Copper	69.97 mnopqrst	6.51	71.12 klmnopqrs	15.95	76.25 efghijklmn	10.41	74.32 hijklmnop	15.82	71.28 klmnopqr	12.36
Streptomycin	1.76 y	97.64	2.05 y	97.57	3.74 y	95.60	1.69 y	98.08	2.74 y	96.63
Control	74.85 ghijklmnop	0.00	84.62 abcd	0.00	85.11 abc	0.00	88.29 a	0.00	81.34 bcdefg	0.00
2003										
Hrp	31.76 x	55.83	37.84 wx	52.56	35.61 wx	52.89	39.65 w	48.64	34.84 wx	52.84
Hrp+Copper	32.19 x	55.24	36.42 wx	54.34	35.31 wx	53.29	36.28 wx	53.01	36.13 wx	51.09
Humic acid	72.79 ijklmnopq	0.00	78.69 cdefghij	1.35	71.87 jklmnoopq	4.93	80.13 bcdefgh	0.00	74.73 ghijklmnop	0.00
HA+Copper	69.12 opqrstuv	3.89	74.63 ghijklmnop	6.44	70.86 lmnopqrs	6.26	76.62 efghijklm	0.76	72.29 jklmnopq	2.15
Copper	63.05 uv	12.33	70.78 lmnopqrs	11.26	68.18 pqrstuv	9.81	69.37 nopqrstuv	10.15	66.01 qrstuv	10.65
Streptomycin	1.34 y	98.13	1.53 y	98.08	1.16 y	98.46	2.07 y	97.31	1.06 y	98.56
Control	71.92 jklmnopq	0.00	79.77bcdefgh	0.00	75.60 fghijklmno	0.00	77.21 efghijkl	0.00	73.88 hijklmnop	0.00

[1]D. S.; Disease Severity, [2]E. C.; Effectiveness of Chemical, *There was an interaction statistically between *disease severities* and *chemicals* and *years* in two years, figures are averages of three replications, each consisting of three shoots ($P<0.01$)

Table 6. Effectiveness of the chemicals on shoot growth of pear cultivars in greenhouse and field conditions in 2002 and 2003

Chemicals	Greenhouse Experiments																			
	2002										*2003*									
	cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey		cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey	
	[1]S.L.* (cm)	[2]E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)
Harpin protein	41.52	0.00	41.48	3.66	45.20	0.00	42.89	3.35	43.83	2.12	41.06	0.00	43.04	0.00	42.08	0.00	42.77	2.15	42.30	0.07
Humic acid	41.61	0.00	44.93	0.00	43.88	0.92	43.96	0.94	45.68	0.00	41.32	0.00	44.03	0.00	42.46	0.00	43.92	0.00	44.07	0.00
Copper salts	40.67	1.73	41.67	3.22	43.14	2.59	43.47	2.05	42.82	4.37	38.61	3.81	41.09	2.35	39.92	4.52	42.06	3.77	41.72	1.44
Streptomycin	42.07	0.00	41.70	3.15	43.62	1.51	43.79	1.32	44.93	0.00	39.09	2.61	42.07	0.02	40.00	4.32	43.99	0.00	40.81	3.59
Water (Control)	41.39	0.00	43.06	0.00	44.29	0.00	44.38	0.00	4478	0.00	40.14	0.00	42.08	0.00	41.81	0.00	43.71	0.00	42.33	0.00
	Field Experiments																			
	2002										*2003*									
	cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey		cv. Ankara		cv. Santa Maria		cv. Williams		cv. Deveci		cv. Riza Bey	
	[1]S.L. (cm)	[2]E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)	S.L. (cm)	E.C. (%)
Harpin protein	77.65	0.00	71.70	0.00	64.84	0.10	61.55	3.48	69.80	0.00	79.92	0.48	76.45	0.00	75.60	1.18	76.18	0.00	76.15	0.00
Humic acid	78.05	0.00	70.38	0.00	65.79	0.00	64.19	0.00	68.63	0.00	80.57	0.00	77.92	0.00	74.87	2.14	76.10	0.00	77.10	0.00
Copper salts…	74.73	3.58	67.48	2.94	61.48	5.28	60.79	4.67	66.96	0.34	78.55	2.19	73.27	3.32	75.03	1.93	74.59	0.06	75.32	0.35
Streptomycin	75.74	2.28	68.74	1.13	66.77	0.00	64.54	0.00	65.65	2.29	79.02	1.60	75.69	0.13	76.07	0.57	75.06	0.00	74.62	1.28
Water (Control)	77.51	0.00	69.53	0.00	64.91	0.00	63.77	0.00	67.19	0.00	80.31	0.00	75.79	0.00	76.51	0.00	74.64	0.00	75.59	0.00

[1]S. L.; Shoot Length, [2]E. C.; Effectiveness of Chemical, *There was not any interaction statistically between variants in two years

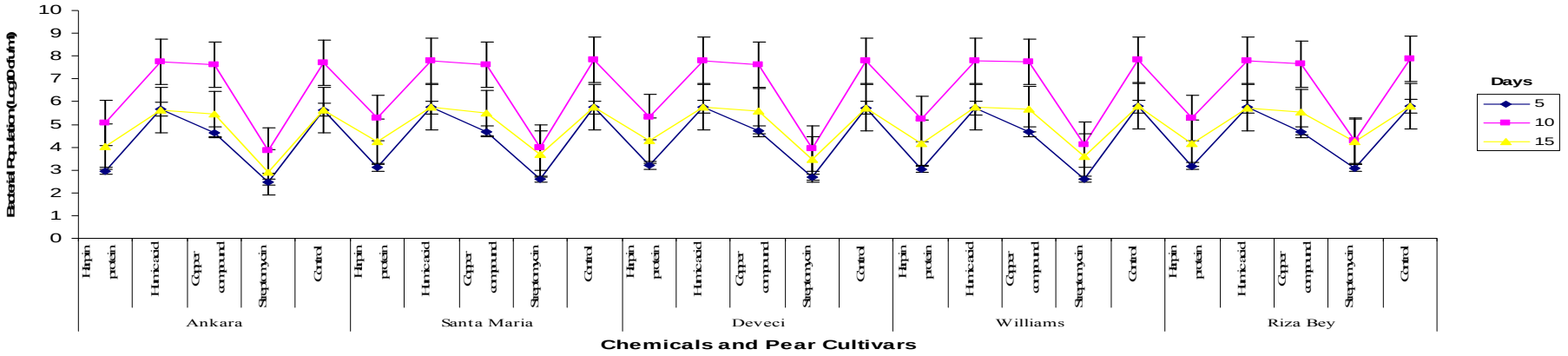

Figure 1. Bacterial population in plant tissues treated by chemicals and inoculated by *E. amylovora* on the 5th, 10th and 15th days

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