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Effect of Antibiotics on the Germination and Root Elongation of Argentine Intensive Crops

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ABSTRACT: For small cheese-making factories, with no effluent treatment plants, whey can be a serious problem and the landfarming technique represents an alternative for its disposal. However, whey may contain antibiotic residues at the Maximum Residue Limits. Therefore, in this work, we evaluated the phytotoxic effect of different concentrations of five antibiotics (enrofloxacin, kanamycin, oxytetracycline, penicillin and tylosin) on the germination frequency and root elongation of five Argentine crops: sunflower (Helianthus annuus), corn (Zea mays), soybean (Glycine max), sorghum (Sorghum bicolor), and wheat (Triticum aestivum). We concluded that the antibiotic concentrations currently allowable for the dairy industry (Maximum Residue Limits) are a potential risk for S. bicolor, corn and G. max crops. Results showed that 0.10 mg/l of enrofloxacin affected sorghum, 0.15 mg/l of kanamycin affected corn and G. max, 0.004 mg/l of penicillin affected corn, G. max and S. bicolor, and that 0.05 mg/l of tylosin affected G. max. Therefore, dumping whey contaminated with antibiotic residues on the soil is not recommended.

Key words: Antibiotics, Phytotoxicity, Seeds, Root elongation, Germination frequency

INTRODUCTION

The use of antibiotics in veterinary medicine represents a widespread practice (Ghava et al., 2015). In milk-producing cattle, antibiotics are used to treat mastitis, metritis, pneumonia, enteritis, hoof diseases, etc. (Sawant et al., 2005; Cabello, 2006; Martínez, 2009; Topp et al., 2013). However, the Argentine legislation prohibits the incorporation of milk contaminated with antibiotic residues above the Maximum Residual Limits (MRLs) into the food chain, whereby, this milk must be removed from the market (Argentine Food Code, 2001). In contrast, milk containing antibiotic residues below the MRLs can be processed in the dairy industry and its effluent can be discarded to the environment (European Community, 2009; Codex Alimentarius, 2010). For small cheese-making factories, with no effluent treatment plants, whey represents a serious problem, which can be solved by landfarming (Mannetje et al., 2008). This technique involves the controlled disposal of effluents on the soil to achieve biological and chemical degradation of organic matter (Silva-Castro et al., 2015). However, its application is limited as long times are required for organic matter biodegradation, because it depends on the microbial composition, the physicochemical properties of the soil and the concentration and type of pollutants (Thassitou and Arvanitoyannis, 2001).

In soils under conventional landfarming fertilised with manure and monitored for two years, Wang and Wang (2015) reported average concentrations of up to 199 µg/kg of tetracycline, 7 µg/kg of chlortetracycline and 11 µg/kg of sulfadimidine. In addition, numerous antibiotic molecules are not metabolised, thus being eliminated in the milk or excreted in the urine and/or feces (Chee-Sanford et al.; 2001; Kumar et al., 2005a,b; Pope et al., 2009; Finley et al., 2013; Topp et al., 2013). These molecules can reach the ground and pass to the groundwater (Pedersen et al., 2003; Aga et al., 2003; Batt et al., 2006; Zhang and Li, 2011) or to watercourses adjacent to livestock production areas (Kolpin et al., 2002; Boxall

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et al 2003; Boxall et al., 2004; Hamscher et al., 2004; Davis et al., 2006; Batt et al., 2006; Aust et al., 2008; Bowman et al., 2011). Therefore, the growth and development of plants (Jjemba, 2002a,b; Liu et al., 2009), the composition of the microbial flora (Mc Cracken Foster, 1993; Chander et al., 2005) and the growth of some native microorganisms (Varnero et al., 2005) can be modified when antibiotic molecules are introduced in the soil.

When manure contaminated with antibiotic residues is dumped on the ground, resistant strains can develop (Knapp et al., 2010). When microorganisms acquire resistance via gene transfer, resistant microbial diseases affecting animals and humans may appear (Thiele Bruhn, 2003; Andersson and Hughes, 2012; Wellington et al., 2013). Therefore, resistant infections are becoming more frequent (Carlet et al., 2011; Finley et al., 2013).

Environmental damage has been assessed by several authors by means of different techniques, including phytotoxicity studies on plant growth and seed germination (Liu et al., 2009). For example, Hillis et al. (2011) studied the effect of some antibiotics on seed germination and root elongation of lettuce (Lactuca sativa), alfalfa (Medicago sativa) and carrot (Daucus carota). Similarly, Bowman et al. (2011) evaluated the toxicity and reduction in intracellular calcium levels due to tetracycline in Arabidopsis. Chen et al. (2011) analysed single and joint toxicity of chloramphenicol with Hg acting on wheat (Triticum aestivum L.), Chinese cabbage (Brassica campestris L.) and corn (Zea mays L.). These authors report a positive correlation between root elongation inhibition and concentrations of pollutants. Liu et al. (2009) analysed the effect of various antibiotics on the growth of rice (Oryza sativa L), turmeric (Cucumis sativus L) and sweet oats (Cichaorium endive), while Migliore et al. (1994, 1998) evaluated the phytotoxicity of sulfadimethoxine, enrofloxacin and oxytetracycline in millet (Panicum miliaceum), peas (Pisum sativum) and maize (Zea mays) plants. Moreover, Jjemba (2002a, b) studied the effect of chloroquine, quinacrine and metronidazole in soybean plants and the soil microbiota. However, no studies have evaluated the potential effects of antibiotics on Argentine widespread crops.

Therefore, the objective of this study was to evaluate the effect of six families of antibiotics (aminoglycosides, beta-lactams, macrolides, quinolones, sulfonamides and tetracyclines) on the germination and root elongation of five Argentine crops (Glycine max, Helianthus annuus, Sorghum bicolor, Triticum aestivum and Zea mays,) to assess their possible phytotoxic effect.

MATERIALS & METHODS

Antibiotics studied: the following antibiotic molecules, representative of each group, were selected: enrofloxacin (quinolones), kanamycin (aminoglycosides), oxytetracycline (tetracycline), penicillin (beta-lactams), and tylosin (macrolides). To assess the possible impact of landfarming with whey containing antibiotic residuals, concentrations equivalent to their MRLs (European Community, 2009; Codex Alimentarius, 2010) were chosen as minimum values for this study. Based on these threshold values, solutions containing the following concentrations in logarithmic scales were prepared: C_0 = 0 MRL, C_1= 1 MRL, C_2 = 10 MRLs, C_3 = 100 MRLs and C_4 = 1000 MRLs ($MRL_{enrofloxacin}$: 0.1 mg/l, $MRL_{oxytretracycline}$: 0.1 mg/l, $MRL_{kanamycin}$: 0.15 mg/l, $MRL_{penicillin}$: 0.004 mg/l, $MRL_{tylosin}$: 0.05 mg/l).

Selected seeds: Glycine max (51%), Zea mays (28%), Triticum aestivum (9%), Helianthus annuus (3%) and Sorghum bicolor (2%) were selected due to its significant production in Argentine (Rosario Stock Exchange, 2015). Crop seeds were provided by Department of Intensive Cultivation of Faculty of Agricultural Sciences, National University of Litoral (Argentine).

Toxicity study on seeds: the guidelines established by the standard protocol to conduct toxicity tests in terrestrial plants were followed (ASTM, 2003; Liu et al., 2009; Hillis et al. 2011; Bowman et al., 2011; Adomas et al., 2013; Ghava et al., 2015). Before starting the treatment, seeds were stored at 4°C for 1 month. After this time, 1500 experimental units of each culture were selected.

For each seed, five replicates of each antibiotic concentration were performed using polystyrene Petri plates (100 x 15 mm). In each plate, 10 seeds were distributed on Whatman cellulose filter. Subsequently, 10 ml of the antibiotic solutions were added to each plate at each concentration (C_0, C_1, C_2, C_3, C_4: 25 plates/antibiotic and seed).

To prevent dehydration, plates were covered with Parafilm M (Sigma-Aldrich) and placed in an incubation chamber at 25°C for 7 days. For germination control, the acceptability criterion was set at 70% (according to the protocol).

Relative frequency of seed germination. The relative frequency of seed germination of each species was calculated at 12-hour intervals during a period of seven days (14 controls). The effect of antibiotic concentration on the germination frequency of each seed was analysed. The stepwise option of the multiple logistic regression models was used:

$$Y = \text{Logistic } L_{ijk} = \beta_0 + \beta_i t_i + \Sigma\, \beta_{0j} Z_j + \varepsilon_{ijk}$$

Where: $Y = \text{Logistic } L_{ijk}$: Logistic function = ln [P_{ijkl}/(1-P_{ijkl})]: P_{ijkl} from germinated seed and (1-P_{ijkl}): probability of non-germinated seeds. β_0: Intercept, $\beta_{0,\ i,\ 0j}$: coefficients estimated by the logistic model, t_i: Effect of time on seed germination (i=12), Z_j: Effect of antibiotic concentration in terms of dummy variable (Z_0: C_1=0, C_2=0, C_3=0 and C_4=0; Z_1: C_1=1, C_2=0, C_3=0 and C_4=0; Z_2: C_1=0, C_2=1, C_3=0 and C_4=0; Z_3: C_1=0, C_2=0, C_3=1 and C_4=0; Z_4: C_1=0, C_2=0, C_3=0 and C_4=1) and ε_{ijk}: residual error of the model. Statistical calculations were performed using Centurion Stat Graphics® software.

Effect of antibiotics on the root elongation of germinated crop seeds. Seven days after the start of the experiment, the root length of each germinated seed was measured in duplicate. Measurements were made from the top of the primary root to the hypocotyl, using a Vernier caliper (accuracy 0.1 mm).

The effect of antibiotic concentration on root elongation was evaluated by means of ANOVA (Centurion Stat Graphics®). Subsequently, Turkey's test was applied to evaluate significant differences for each antibiotic concentration.

RESULTS & DISCUSSION

Relative frequency of seed germination: Statistical results ("χ^2" and "p" value) from the application of the stepwise option of the logistic regression model, which predicts germination frequencies for different antibiotic concentrations, are shown in Table 1. These results show that almost all the seeds analysed were affected by enrofloxacin (except for Zea mays) and penicillin (except Helianthus annuus). The other antibiotics affected a smaller number of seeds (Table 1).

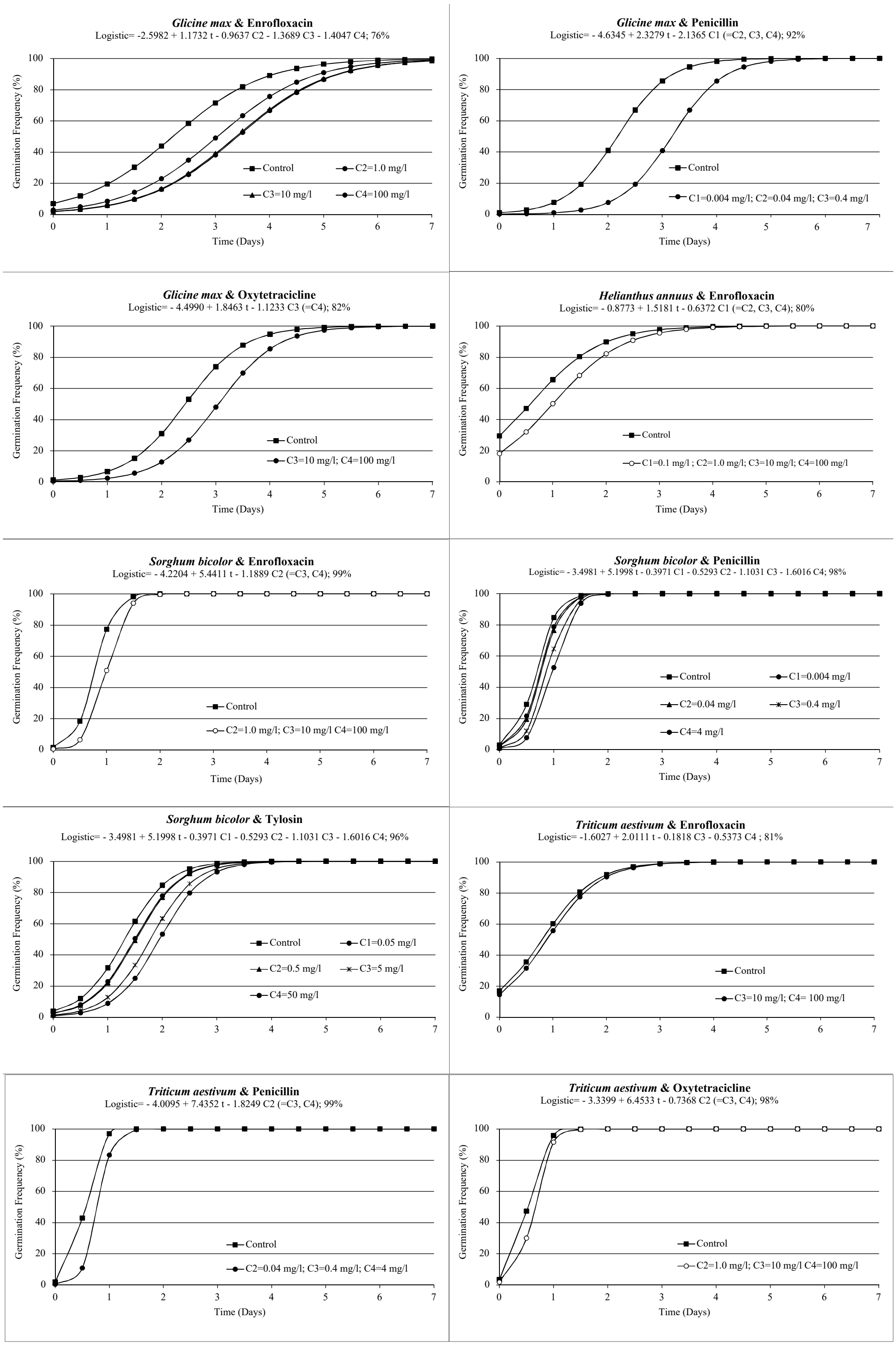
The effects of each antibiotic concentration on the germination frequencies of seeds selected by the stepwise option are shown in Fig. 1. In all the cases, the antibiotics caused a delay in the germination frequencies of seeds, showing a phytotoxic action on crop growth. In addition, the logistic equations are shown in Fig. 1. The time affected the germination frequencies significantly (positive coefficients "β_i"), while the antibiotic concentration negatively affected the germination frequencies, demonstrating an inhibitory effect (negative coefficients "β_{0j}").

Fig. 1 show that 0.004 mg/l of penicillin, 1.0 mg/l of enrofloxacin and 10 mg/l of oxytetracycline affected the germination frequency of Glycine max and that 0.1 mg/l of enrofloxacin affected the germination frequency of Helianthus annuus. The germination frequency of Sorghum bicolor decreased with 0.004 mg/l of penicillin and 0.05 mg/l of tylosin, values equivalent to their respective MRLs. Higher values of enrofloxacin (1 mg/l) were necessary to affect the germination frequency of this crop. Triticum aestivum seeds were affected by 0.4 mg/l of penicillin, 1 mg/l of oxytetracycline and 10 mg/l of enrofloxacin. Zea mays seeds were more resistant to antibiotic residues, since 100 mg/l of oxytetracycline and 4 mg/l of penicillin were necessary to affect their germination frequency.

With the aim to visualize this inhibitory action of antibiotics, Fig. 2 (1a: treated group and 1b: control

Table 1. Relative frequency of seed germination - Statistical results from the application of the stepwise option of the logistic regression model for different antibiotic concentrations

Seed	Antibiotic	Time		Concentration	
		¡^{0} valor	P valor	¡^{0} valor	p-valor
Glicine max	Enrofloxacin	1316	0.0001	97.9	0.0001
	Oxytetracycline	812	0.0001	32.9	0.0001
	Penicillin	935	0.0001	90.2	0.0001
Helianthus annuus	Enrofloxacin	971	0.0001	16.3	0.0027
Sorghum bicolor	Enrofloxacin	876	0.0001	12.7	0.0004
	Penicillin	2066	0.0001	29.4	0.0001
	Tylosin	2219	0.0001	95.0	0.0001
Triticum aestivum	Enrofloxacin	874	0.0001	6.79	0.0334
	Oxytetracycline	1753	0.0001	6.82	0.0090
	Penicillin	808	0.0001	20.3	0.0001
Zea mays	Oxytetracycline	1184	0.0001	10.4	0.0013
	Penicillin	1172	0.0001	11.9	0.0005


Glicine max & Enrofloxacin
Logistic= -2.5982 + 1.1732 t - 0.9637 C2 - 1.3689 C3 - 1.4047 C4; 76%
Germination Frequency (%)
Time (Days)
Control
C2=1.0 mg/l
C3=10 mg/l
C4=100 mg/l
Glicine max & Penicillin
Logistic= - 4.6345 + 2.3279 t - 2.1365 C1 (=C2, C3, C4); 92%
Germination Frequency (%)
Time (Days)
Control
C1=0.004 mg/l; C2=0.04 mg/l; C3=0.4 mg/l
Glicine max & Oxytetracicline
Logistic= - 4.4990 + 1.8463 t - 1.1233 C3 (=C4); 82%
Germination Frequency (%)
Time (Days)
Control
C3=10 mg/l; C4=100 mg/l
Helianthus annuus & Enrofloxacin
Logistic= - 0.8773 + 1.5181 t - 0.6372 C1 (=C2, C3, C4); 80%
Germination Frequency (%)
Time (Days)
Control
C1=0.1 mg/l ; C2=1.0 mg/l; C3=10 mg/l; C4=100 mg/l
Sorghum bicolor & Enrofloxacin
Logistic= - 4.2204 + 5.4411 t - 1.1889 C2 (=C3, C4); 99%
Germination Frequency (%)
Time (Days)
Control
C2=1.0 mg/l; C3=10 mg/l C4=100 mg/l
Sorghum bicolor & Penicillin
Logistic= - 3.4981 + 5.1998 t - 0.3971 C1 - 0.5293 C2 - 1.1031 C3 - 1.6016 C4; 98%
Germination Frequency (%)
Time (Days)
Control
C1=0.004 mg/l
C2=0.04 mg/l
C3=0.4 mg/l
C4=4 mg/l
Sorghum bicolor & Tylosin
Logistic= - 3.4981 + 5.1998 t - 0.3971 C1 - 0.5293 C2 - 1.1031 C3 - 1.6016 C4; 96%
Germination Frequency (%)
Control
C1=0.05 mg/l
C2=0.5 mg/l
C3=5 mg/l
C4=50 mg/l
Triticum aestivum & Enrofloxacin
Logistic= -1.6027 + 2.0111 t - 0.1818 C3 - 0.5373 C4 ; 81%
Germination Frequency (%)
Control
C3=10 mg/l; C4= 100 mg/l
Triticum aestivum & Penicillin
Logistic= - 4.0095 + 7.4352 t - 1.8249 C2 (=C3, C4); 99%
Germination Frequency (%)
Time (Days)
Control
C2=0.04 mg/l; C3=0.4 mg/l; C4=4 mg/l
Triticum aestivum & Oxytetracicline
Logistic= - 3.3399 + 6.4533 t - 0.7368 C2 (=C3, C4); 98%
Germination Frequency (%)
Time (Days)
Control
C2=1.0 mg/l; C3=10 mg/l C4=100 mg/l

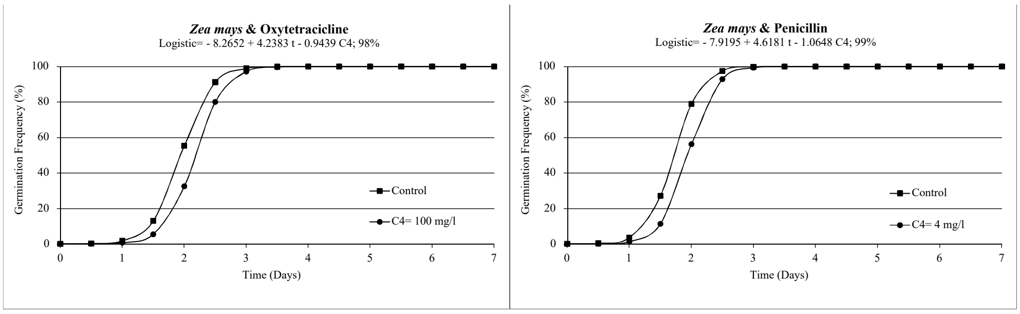


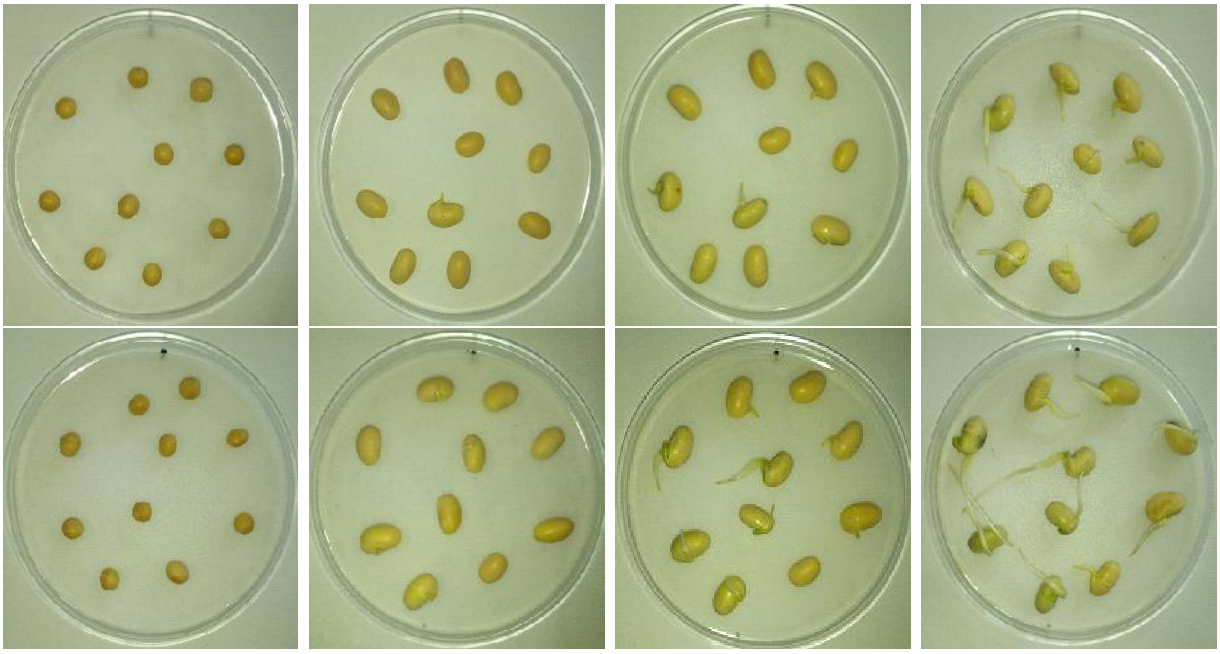
Fig. 1. Relative frequency of seed germination - Effects of antibiotics concentration on the germination frequencies of seeds

group) is presented. This image shows that low concentrations of penicillin (0.004 mg/l) affect germination frequency of Glycine max comparing with the control (free-antibiotic) , on second day (1 vs. 4 germinated seeds) and fourth day (4 vs. 8 germinated seeds). By the fifth day, all the seeds have germinated in both groups.

To summarise, it can be established that antibiotic concentrations equivalent to the MRLs cause a phytotoxic effect on the germination rate of some crops, with 0.1 mg/l of enrofloxacin affecting H. annuus seeds, 0.004 mg/l of penicillin affecting G. max and S. bicolor, and 0.05 mg/l of tylosin affecting S. bicolor.

Effect of antibiotics on the root elongation of germinated crop seeds: The mean values, standard deviations and statistical parameters (“F” and “p” values) calculated with ANOVA for the antibiotics that affected seeds are shown in Table 2. Significant differences obtained by applying Turkey’s test are also presented.

For Glycine max, all the antibiotics tested caused a phytotoxic effect. Kanamycin, penicillin and tylosin showed toxicity at their MRLs ($MRL_{kanamycin}$: 0.15 mg/l, $MRL_{penicillin}$: 0.004 mg/l, $MRL_{tylosin}$: 0.05 mg/l), whereas 1 mg/l of oxytetracycline and 10 mg/l enrofloxacin were necessary to affect root elongation. Regarding



Day 0 Day 2 Day 4 Day 5

Fig. 2. Effect of 0.004 mg/l penicillin on seed germination frequency of Glycine max: (a) treated group, (b) control group

Table 2. Effect of antibiotics on the root elongation of germinated crop seed - Main values, standard deviations and statistical parameters calculated with ANOVA for the antibiotics that affected seeds

Seed	Antibiotic	Concentration (MRL) 0	MRL	10 MRL	100 MRL	1000 MRL	Value "F"	Value "p"
Glicine max	Enrofloxacin	$2,1_a \pm 0,1$	$2,1_a \pm 0,1$	$1,9_{a,b} \pm 0,1$	$1,6_{b,c} \pm 0,1$	$1,4_c \pm 0,1$	7,92	0,0001
	Kanamycin	$1,8_a \pm 0,1$	$1,4_b \pm 0,1$	$1,5_b \pm 0,1$	$1,4_b \pm 0,1$	$1,4_b \pm 0,1$	3,74	0,0060
	Oxytetracycline	$3,6_a \pm 0,2$	$3,2_{a\,b} \pm 0,2$	$2,9_{b\,c} \pm 0,2$	$2,3_{c\,d} \pm 0,2$	$1,6_d \pm 0,2$	19,12	0,0001
	Penicillin	$2,7_a \pm 0,2$	$1,7_b \pm 0,2$	$1,6_b \pm 0,2$	$1,9_b \pm 0,2$	$1,8_b \pm 0,2$	5,62	0,0003
	Tylosin	$3,0_a \pm 0,1$	$2,5_b \pm 0,1$	$2,4_b \pm 0,2$	$2,5_b \pm 0,2$	$1,8_c \pm 0,2$	9,42	0,0001
Helianthus annuus	Enrofloxacin	$2,3_a \pm 0,1$	$2,1_{a,b} \pm 0,1$	$2,1_{a,b} \pm 0,1$	$1,7_b \pm 0,1$	$1,7_b \pm 0,1$	6,54	0,0001
	Oxytetracycline	$2,9_a \pm 0,2$	$2,9_a \pm 0,2$	$2,7_a \pm 0,2$	$2,3_a \pm 0,2$	$1,6_b \pm 0,2$	6,76	0,0001
Sorghum bicolor	Enrofloxacin	$7,4_a \pm 0,2$	$6,2_b \pm 0,2$	$3,9_c \pm 0,2$	$2,8_d \pm 0,2$	$2,8_d \pm 0,2$	110,91	0,0001
	Kanamycin	$5,7_a \pm 0,3$	$5,1_a \pm 0,3$	$5,1_a \pm 0,3$	$2,8_b \pm 0,3$	$1,4_b \pm 0,2$	61,41	0,0001
	Oxytetracycline	$6,8_a \pm 0,2$	$6,0_a \pm 0,2$	$5,3_a \pm 0,2$	$1,9_b \pm 0,2$	$2,0_b \pm 0,2$	41,97	0,0001
	Penicillin	$8,3_a \pm 0,3$	$6,2_b \pm 0,3$	$6,6_b \pm 0,3$	$6,6_b \pm 0,3$	$6,4_b \pm 0,3$	6,53	0,0001
Triticum aestivum	Enrofloxacin	$5,4_a \pm 0,2$	$4,3_a \pm 0,2$	$3,9_a \pm 0,2$	$2,4_b \pm 0,2$	$1,9_b \pm 0,2$	38,50	0,0001
	Kanamycin	$5,1_a \pm 0,2$	$5,4_a \pm 0,2$	$5,3_a \pm 0,2$	$2,3_b \pm 0,2$	$1,1_b \pm 0,2$	78,38	0,0001
	Oxytetracycline	$5,1_a \pm 0,1$	$5,2_a \pm 0,1$	$3,5_b \pm 0,1$	$1,4_c \pm 0,1$	$0,8_c \pm 0,1$	175,59	0,0001
	Penicillin	$6,4_a \pm 0,3$	$6,0_a \pm 0,3$	$5,7_{a,b} \pm 0,3$	$5,1_b \pm 0,3$	$5,1_b \pm 0,3$	3,78	0,0078
Zea mays	Enrofloxacin	$1,8_a \pm 0,1$	$1,8_a \pm 0,1$	$1,7_a \pm 0,1$	$1,7_a \pm 0,1$	$1,5_b \pm 0,1$	3,33	0,0117
	Kanamycin	$1,4_a \pm 0,1$	$1,2_b \pm 0,1$	$1,2_b \pm 0,1$	$1,1_{b,c} \pm 0,1$	$0,9_c \pm 0,1$	7,45	0,0001
	Oxytetracycline	$2,9_a \pm 0,1$	$3,1_a \pm 0,1$	$2,9_a \pm 0,1$	$1,9_b \pm 0,1$	$1,7_b \pm 0,1$	37,15	0,0001
	Penicillin	$2,0_a \pm 0,1$	$1,6_b \pm 0,1$	$1,7_b \pm 0,1$	$1,7_b \pm 0,1$	$1,7_b \pm 0,1$	3,47	0,0091

a, b, c: Different subscripts values in the same row indicate significant differences at a level of $p<0.05$
$MRL_{enrofloxacin}$: 0.1 mg/l, $MRL_{kanamycin}$: 0.15 mg/l, $MRL_{oxytretracycline}$: 0.1 mg/l, $MRL_{penicillin}$: 0.004 mg/l, $MRL_{tylosin}$: 0.05 mg/l

Helianthus annuus, the inhibitory effect was observed at 10 mg/l of enrofloxacin and 100 mg/l of oxytetracycline, higher values than MRLs.

Sorghum bicolor root elongation was affected by 0.1 mg/l of enrofloxacin (MRL), 0.004 mg/l of penicillin (MRL), 15 mg/l of kanamycin and 10 mg/l of oxytetracycline. It must be noted that oxytetracycline caused a decrease in root length greater than 85%.

Triticum aestivum root elongation was affected by 1 mg/l of oxytetracycline, 10 mg/l of enrofloxacin, 15 mg/l of kanamycin and 0.4 mg/l of penicillin, higher values than MRLs. It should be noted that the presence of 10 mg/l of enrofloxacin and 15 mg/l of kanamycin caused a decrease in root length greater than 50%. Ghava et al. (2015) studied the effects of amoxicillin, levofloxacin and tetracycline over three wheat species (Triticum aestivum) and did not observe phytotoxic effect of these antibiotics (except for tetracycline).

For Zea mays, inhibitory effects were observed at low concentrations of kanamycin (0.15 mg/l) and penicillin (0.004 mg/l) but at high values of oxytetracycline (10 mg/l) and enrofloxacin (100 mg/l). In summary, oxytetracycline affected the root elongation of all seeds studied. This is in agreement with the results of Hillis et al. (2011), who reported inhibitory effect of oxytetracycline in root elongation of Lactuca sativa, Medicago sativa and Daucus carota. When comparing the germination frequency of seeds (Table 1) with their root elongation (Table 2), we found that the second parameter was more sensitive, as also noted by Ghava et al. (2015) and Basset et al. (2002).

In order to appreciate the impacts of low antibiotic concentrations on the roots elongation, the effect of penicillin (0.004 mg/l) on Glycine max was chosen. In Fig. 3 is displayed that penicillin produced a significant decrease in the length of the roots (Fig. 3a) compared

Fig. 3. Effect of penicillin (0.004 mg/l) on root elongation of Glycine max determined the seventh day of treatment. (a) Control group, (b) treated group

to free-antibiotics seeds (Fig. 3b) at the seventh day of treatment.

In summary, our results showed that low concentrations of some antibiotics affect the root elongation of the main Argentine crops: 0.15 mg/l of kanamycin, 0.004 mg/l of penicillin and 0.05 mg/l of tylosin affected Glycine max seeds; 0.1 mg/l of enrofloxacin and 0.004 mg/l of penicillin affected Sorghum bicolor, and 0.15 mg/l of kanamycin and 0.004 mg/l of penicillin affected Zea mays. Therefore, the landfarming technique for disposal of whey contaminated with antibiotics at the MRLs values established by the law should be avoided.

In order to avoid the environmental impact of these antibiotics, heat treatment or photocatalytic processes of contaminated whey should be evaluated. Studies in milk indicate that sterilization process (120°C-20min) produces significant losses in the antimicrobial activity of penicillin (Zorraquino et al., 2008, Roca et al., 2011), kanamycin (Zorraquino et al., 2009), tylosin (Zorraquino et al., 2011), except enrofloxacin (Roca et al., 2010). For degradation of quinolones residues in water, Sirtori et al. (2009) suggest a solar photocatalytic processes.

CONCLUSIONS

Due to the possibility of using the landfarming technique for the treatment of contaminated whey, here we evaluated the effect of different antibiotics on the germination frequency and root elongation of some Argentinean crops. We concluded that values equivalent to the Maximum Residue Limits cause phytotoxic effects. Results showed that 0.1 mg/l of enrofloxacin affects the germination of Sorghum bicolor, that 0.15 mg/l of kanamycin affects Zea mays and Glycine max, that 0.004 mg/l of penicillin affects Zea mays, Glycine max and Sorghum bicolor, and that 0.05 mg/l of tylosin affects Glycine max.

These studies show that antibiotic concentrations acceptable for the dairy industry can cause a decrease in the germination frequency and/or a reduction in the root elongation of Glycine max Sorghum bicolor and Zea mays, widely spread crops in Argentine. Helianthus annuus and Triticum aestivum were more resistant. Therefore, the discharge of effluents from the dairy industry contaminated with antibiotics in the soil constitutes an environmental problem.

In order to improve these findings, other aminoglycosides, beta-lactams, macrolides and quinolones should be investigated. It would also be appropriate to assess potential ecotoxicological effects of antibiotics on other biomarkers such as bacteria (Vibrio fischeri), algae (Chlorella vulgaris, Cyclotella meneghiniana, Lemna gibba, Pseudokirchneriella subcapitata), invertebrates (Artemia salina, Ceriodaphnia dubia, Daphnia magna), and fish (Danio rerio, Lepomis punctatus, Lepomis macrochirus, Oncorhynchus mykiss, Salmo trutta, Salvelinus fontinalis, Salvelinus namaycush) to select the most suitable biosensor to be used in the future to control the environmental impact of antibiotics.

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