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Assessment of wastewater toxicity from the pharmaceutical industry of salicylic acid and paracetamol on the roots of a plant species: *Phaseolus vulgaris*

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Abstract: Pharmaceutical compounds are currently a group of emerging environmental contaminants where their presence is reported in all ecosystems (water, soil, etc). Pharmaceutical compounds are currently a group of emerging environmental contaminants where their presence is reported in all ecosystems (water, soil, etc.). Their effects often result in essential metabolic disturbances, which consequently reflect on all vital functions of organisms, including plants which are non-target species. During their growth periods, plants can absorb and accumulate many xenobiotics, including pharmaceutical compounds that are able to cross the root walls. Therefore, in the present study, to evaluate the toxic effects of salicylic Acid (SA) and Paracetamol (PAR) on beans (*Phaseolus vulgaris*), the roots of the study plant were investigated. The root length showed a significant reduction with an increase in the concentration of PAR, whereas with the highest concentration of SA there was an increase in the length of the roots. On increasing the concentration of both PAR and SA, a gradual increase in the fresh weight of roots was observed compared to the control group. After exposure to the different concentrations, PAR and SA treatments caused damage to the protein synthesis. In addition, significant stimulation of proline synthesis was recorded after treatment with PAR, in comparison with the value obtained in the presence of SA at the highest concentration. Lipid peroxidation supported by a significant increase in MDA and H2O2 levels, particularly for PAR treatments, is also observed. Furthermore, the evaluation of the respiratory activity of the isolated bean roots showed oxygen consumption related to the PAR concentrations. This consumption is much higher in the presence of SA. Finally, the findings of the current work indicate those pharmaceutical products and their residues (such as PAR

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and SA), released into the environment in large quantities and without treatment, can produce morphological, biochemical and metabolic changes in the roots of non-target plant species such as beans. Which consequently causes seriously disrupts root functioning.

Keywords: Salicylic acid; Paracetamol; *Phaseolus vulgaris*; Root; Stress.

1 Introduction

The rapid evolution of the density of the population in the world is one of the capital causes of the explosion of the needs for pharmaceutical products (Aruguman, 2007, Redshaw et al., 2013). Thus, the major interest of the pharmaceutical industry has focused on the persistence and chemical stability of these compounds in the human body in order to effectively exert their therapeutic effects (Zhou et al., 2004). Due to the particularity of the properties of these pharmaceutical compounds (significant presence in all ecosystems) and their potential toxicity, these compounds constitute the most dangerous pollutants responsible for the degradation of the environment, particularly for non-target organisms (Lopez-Pacheco et al., 2019). The current Covid-19 pandemic has considerably stimulated the consumption of large quantities of pharmaceutical products such as non-steroidal anti-inflammatory drugs, where the most used are acetylsalicylic acid and paracetamol (Conaghan, 2012; Guzik et al., 2013; Harshkova and Aksmann, 2019). Aspirin or acetyl-salicylicacid belongs to the family of salicylates, it is one of the most frequently prescribed drugs and Paracetamol is a widely used analgesic in medicine. The main sources of pollution by these pharmaceuticals are wastewater where they are present in their original or slightly modified form Addison et al. (1984); Heberer (2002); Bellver-Domingo et al. (2017). The wastewater treatment methods currently used remain very insufficient and are not very effective for the elimination of these products (Zhang et al., 2014; Ebele et al., 2017; Masud et al., 2020). Currently, the study of the effects of these compounds on aquatic ecosystems has revealed many physiological effects (malformations) (Amina et al., 1997; Kramer et al., 1998). At the same time, very little research has looked at the effects of these compounds on plant organisms as non-target species.

Many plant species are able to fix (absorb) these pharmaceutical contaminants using some of their metabolic processes such as phytoremediation (He et al., 2018; Nivala et al., 2019; Zhang et al., 2018; Marsidi et al., 2016). Thus, depending on their concentrations and their physico-chemical properties, these two compounds (acetylsalicylic acid and paracetamol) are capable of causing oxidative stress in plants by disturbing many physiological and biochemical parameters such as metabolic contents (proteins, phytohormones, carbohydrates), enzymes activities, membrane fluidity (Yan et al., 2016; Landa et al., 2018; Lutterbeck et al., 2015; Svobodníkova et al., 2019; Carter et al., 2015; Yu et al., 2017; Kummerova et al., 2016). Therefore, despite the presence of significant quantities of acetylsalicylic acid and paracetamol in the environment, the evaluation of their toxic effects and the metabolic process by which their retentionis carried out by plants remains unclear.

In the present study, we examined the behavior of a *Phaseolus vulgaris* plant after exposure to these two widely used compounds whose future predictions suggest a significant increase in their production: acetylsalicylic acid and paracetamol. We used a series of tests that allow us to examine the potential toxic effects of the two xenobiotics on root length, fresh weight, protein synthesis, respiratory activity of isolated roots, proline as a stress marker, ROS synthesis, H2O2 and MDA content.

2 Materials and Methods

2.1. Chemicals:

Paracetamol (PAR) and Salicylic Acid (SA) were purchased from the central pharmacy in Annaba, Algeria, and used without any other treatment. All other chemicals used in our research were purchased from Sigma-Aldrich, USA. Five different concentrations of PAR (mg/L) and SA (mM) 100, 200, 300, 400, 500 and 0 (control) were prepared from the drug available in the market.

2.2. Seed cultivation and treatments:

The plant material used was beans (*Phaseolus vulgaris*). Bean seeds are provided from the local market. Seeds of uniform weight were disinfected with HgCl2 (0.5%) for 10 min, then washed thoroughly with distilled water. The bean seeds are then germinated in Petri dishes 10 cm in diameter (10 seeds per Petri dish) and placed in a controlled climatic chamber set at a temperature of 25 °C and a relative humidity of 85% for 48 hours. After 3 days of seed germination (12/12 hours of photo-period), the seedlings were transplanted into cultivation containers with a nutrient solution. The seeds are then treated with 3 ml of solutions composed of different concentrations of PAR and SA for 7 days. Germination is considered positive when the length of the roots is equal to 5 mm (Kaur et al., 1998). On the 8th day, the parameters measured are: Root length, Fresh weight, Protein, Proline, MDA, H2O2 and Root respiration.

2.3. Fresh Weight:

Five seeds were randomly selected from each treatment and weighed to find out the fresh weight.

2.4. Root Length:

The length of the roots was measured as described by Wang et al. (2009).

2.5. Total Protein:

Level of soluble proteins, were determined according to the methodology described in Bradford (1976).

2.6. Proline:

Free proline content was determined spectrophotometrically by measuring the change in absorbent at 520 nm (Khalid et al., 2001). The content of proline was expressed in mg/g FW.

2.7. MDA and H2O2 content:

Lipid peroxidation was measured spectrophotometrically and expressed as malondialdehyde (MDA) content according to Zezulka et al. (2019). The hydrogen peroxide content roots were determined according to Velikova et al. (2000). Root samples were homogenized with TCA in an ice bath and then centrifuged (15000g X 10 min). The H2O2 content was measured by spectrophotometry at the wavelength of 390 nm.

2.8. Root Respiration:

Respiratory activity of isolated roots of *Phaseolus vulgaris*, was measured according to Djebar and Djebar (2000). Respiratory activity was monitored using a Clark oxygen electrode (Hansatech Ltd, Kinj'sLym, U, K) coupled to a computer.

2.9. Statistical analysis:

Data obtained were analyzed statistically using one way ANOVA and Dunnett test. Values with less than p 0.05 were considered significant statistically. All statistical analyses were carried out using Minitab version 19.0.

3 Results

3.1. Effects of PAR and SA on root length:

Exposure of *P. vulgaris* roots to PAR concentrations causes a strong reduction in root length from 5.2 to 0.7 cm. This reduction is proportional to the increase in PAR concentrations (87% at 500 mg/L). At the same time, a significant increase (65%) in the length of the roots of *P. vulgaris* is recorded in the presence of SA (500 mM) (Fig. 1).

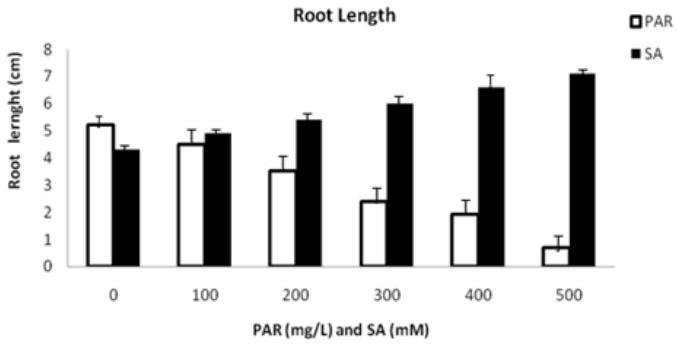

Figure 1: Effects of PAR (mg/L) and SA (mM) on total length of roots (cm) of Phaseolus vulgaris seedlings ($p \leq 0.05$).

3.2. Effects of PAR and SA on Fresh Weight:

The results obtained indicate that treatment with PAR has a positive effect on the fresh weight of the roots of P. vulgaris (increase of 73%). At the same time, the increase in the fresh weight content was observed with the application of SA from the concentration of 200 mM, moreover, it is observed that when the SA concentration increases the fresh weight content also increases, (177% at 500 mM) (Fig. 2).

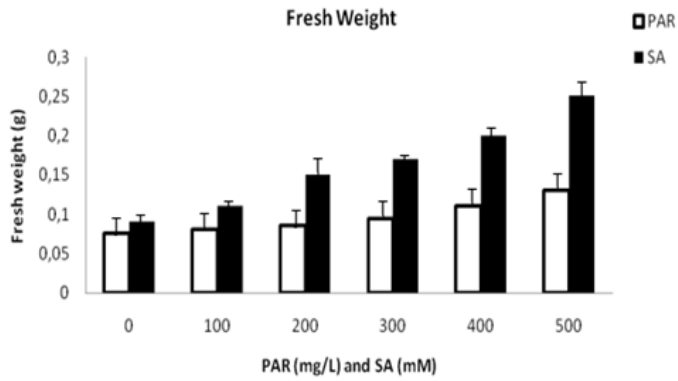

Figure 2: Effects of PAR (mg/L) and SA (mM) on Fresh weight (g) of roots of Phaseolus vulgaris ($p \leq 0.05$).

3.3. Effects of PAR and SA on total protein:

PAR influenced SP content in bean roots, especially at the highest concentration (Fig. 3) where there is a 45% reduction in protein content. A similar evolution of the SP content is observed in the roots treated with SA. Indeed, after exposure to different concentrations of SA a much smaller reduction is recorded. SA at 500 mM reduced the total protein synthesis in bean roots by 23%. This is approximately 2 times less than that obtained in the roots treated with PAR.

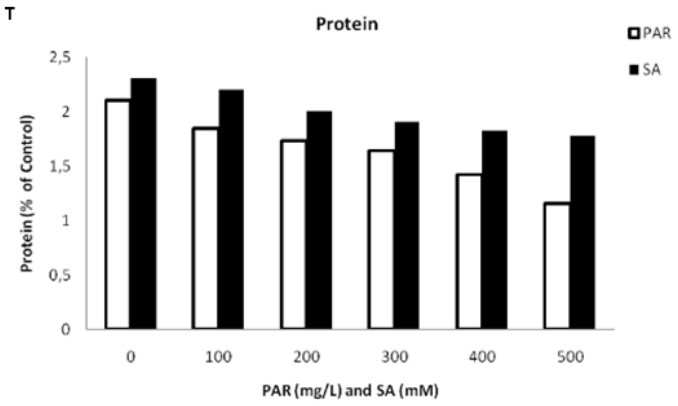

Figure 3: Effects of PAR (mg/L) and SA (mM) on content of proteins (% of control) of roots of *Phaseolus vulgaris* ($p \leq 0.05$).

3.4. Effects of PAR and SA on free proline:

Proline is a very important marker of oxidative stress in plants. It is an aminoacid that plays an important role in the regulation of cellular osmolality. In Figure 4, an increase in proline content was observed in bean roots after application of the two xenobiotics (PAR and SA). However, this effect is greater in roots exposed to SA (x6 compared to controls) than those exposed to PAR (x2.2) compared to controls) (Fig. 4).

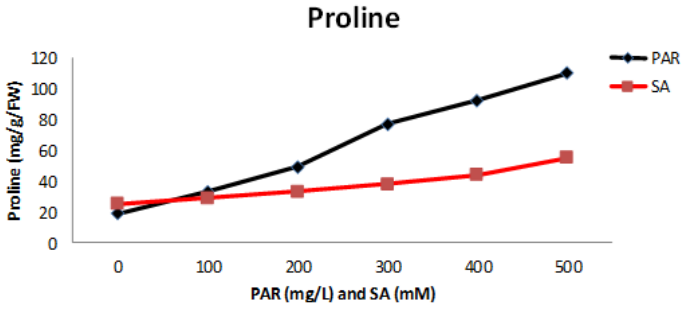

Figure 4: Effects of PAR (mg/L) and SA (mM) on content of proline (mg/g.FW) of roots of *Phaseolus vulgaris*.

3.5. Effects of PAR and SA on Content of MDA and H2O2:

A second important biomarker of oxidative stress in plants is the rate of hydrogen peroxide, which is a compound capable of crossing cell membranes. Our results show that treatment with PAR induces an increase in H2O2 content, (x 2.3, Table 1). In addition, the malondialdehyde (MDA) content (indicative parameter of the level of lipid peroxidation) also increases (x 1.75, Table 1). Exposure of roots to SA concentrations also resulted in H2O2synthesis, but much less than observed with PAR. Indeed, this one is about 2 times less important (14.9-7.2). It was also observed that the application of SA on *Phaseolus* roots caused a slight increase (from15.2 to 19.5 µg/g WF) in MDA content (Table 1).

Table 1. Effects of PAR (mg/L) and SA (mM) on the content of Malondialdehyde (µg/g FW) and hydrogen peroxide (µmol/g FW) of roots of *Phaseolus vulgaris*.

DOL (mg/L)	H2O2 (µmol/g.FW)	MDA (µg/g.FW)	SA(mM)	H2O2 (µmol/g.FW)	MDA (µg/g.FW)
0	11.5 ± 0.1	22.1 ± 0.001	0	9.1 ± 0.002	15.2 ± 0.001
10	14.4 ± 0.001	23.9 ± 0.002	10 ± 0.01	9.5 ± 0.002	16.6 ± 0.02
20	18.8 ± 0.02	26.8 ± 0.004	20 ± 0.02	10.3 ± 0.007	17.1 ± 0.005
300	22.1 ± 0.002	29.1 ± 0.008	300 ± 0.5	11.4 ± 0.004	17.7 ± 0.004
400	24.2 ± 0.03	34.3 ± 0.002	400 ± 0.09	14 ± 0.02	18.6 ± 0.002
500	26.4 ± 0.01	38.7 ± 0.001	500 ± 0.02	16.3 ± 0.06	19.5 ± 0.007

3.6. Effects of PAR and SA on bean root respiratory activity:

The respiratory activity of the roots reflects the state of the energy metabolism of these organs. It is assessed by measuring the respiration. In this way, the results obtained in our work show that exposure to PAR induce a low stimulation of the respiratory activity of bean roots as a function of time (56 nmol/ml of O2 consumed compared to control roots). In parallel, the application of SA strongly stimulates this activity (179 nmol/ml of O2 consumed, Fig. 5). It is clearly apparent that treatment with SA significantly activates the respiratory activity of bean roots by about 3 times higher compared to that exposed to PAR.

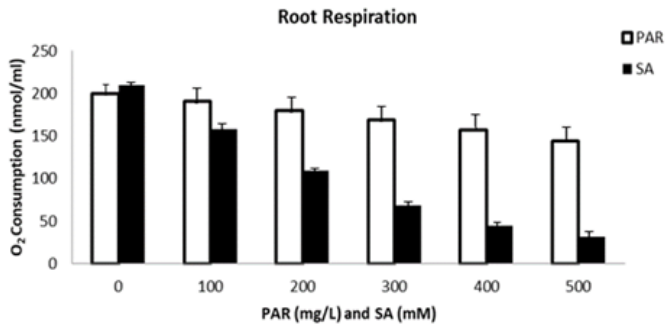

Figure 5: Effects of PAR (mg/L) and SA (mM) on roots respiration (nmol. O2/ml) of *Phaseolus vulgaris* ($p \leq 0.001$).

4 Discussion

Contamination of the environment by drugs today represents a main source of pollution due to their toxic potential based on their physical and chemical properties, in particular for non-target organisms such as plants or marine organisms (Chagra et al., 2007; Rouabhi et al., 2006; Gasmi et al., 2016). In the present study, each of these two xenobiotics applied had a different effect on the different morphological and biochemical parameters of the experimental model used, *P. vulgaris*. Root length can be an important external factor of internal plant metabolic disturbance (Soukup et al., 2002; Rachid et al., 2008). The exposure to PAR affected on the length of the roots, so the observed inhibition would be responsible for the reduction of the roots which are shorter. Treatment with PAR would be able to strongly inhibit cellular mitotic activity. In contrast, exposure to SA causes a strong stimulation of root length, these results are identical to those obtained by Kummerova et al. (2016). The root morphological modifications observed following the exposure of plants to the two xenobiotics, can induce changes or even disturbances in the metabolic processes of adaptation/resistance to various stressful situations. Similar results have been reported in the work of Nishida et al. (2005) and Bambridge et al. (1995). Constant increase in PAR and SA concentrations showed significant changes on bean root fresh weight. This effect is much greater in roots exposed to SA. This result is contrary to that reported by 17 which shows that treatment with pharmaceuticals has no effect on growth in wheat (Rouabhi, 2007). During the growth of plants, proteins play a key role through their many interventions in all vital metabolic processes. After germination of bean seeds exposed to the two xenobiotics, the seedlings will develop according to an exponential phase of growth where their metabolic processes, including protein synthesis reaches their maximum activity.

Results of the current study showed that the protein content of isolated roots decreases as a function of exposure time and with the two xenobiotics used. These findings are in agreement with the results of the work of Rouabhi et al. (2006), Wu and Von (2002), and Liao et al. (2000). In plants, proline plays a very important role as a marker of oxidative stress. This compound is essential for the enzymatic functioning of plants by controlling cellular osmolarity, particularly in stressful situations (Salim et al., 2016; Liu et al., 2014; Matzke et al., 2009). Also,the present study showed a clear increase in proline in roots exposed to PAR. Contrary to the observations noted under exposure to SA where the changes recorded are less significant. In this way, many studies confirm our results (Sousa et al., 2020; Misra and Gupta, 2005; Jogeswar et al., 2006; Pawłowska et al., 2019). The production of ROS in isolated bean roots is much higher under exposure to PAR than SA. These modifications reflect the importance of the cellular damage which affects the bean roots and which causes disturbances in the major function of the membrane like permeability. These physiological and biochemical modifications of the cell membranes are due to a destruction of the membrane components (lipid peroxidation)that observed by the increase in the content of MDA (Souiki et al., 2008; Zezulka et al., 2019). It seems that PAR and SA act at the level of cell membranes via the synthesis of hydrogen peroxide produced by oxidative stress due to the two xenobiotics. However, it is to note that even the mode of action of these two xenobiotics seems identical; the impact of SA appears less toxic than the PAR.

In this work has also been noticed that the effects of the two xenobiotics on lipid peroxidation were important, particularly for PAR (very strong inhibition of respiratory activity). This effect reflects the triggering of oxidative stress which is also at the origin of the significant ROS synthesis recorded (Gonzalez et al., 2008). These ROS are mainly synthesized by the mitochondria and mainly by the mitochondrial respiratory chain under stress conditions (Toualbia et al., 2017; Yamamoto et al., 2002, 2003). Overall, the findings of this research are in agreement with those

results that obtained by Bouchlaghem et al. (2011) with metal dusts as a xenobiotic (Zouainia et al., 2016). Exposure to PAR of isolated roots can affect the membrane structure of mitochondria by reducing energy parameters such as membrane potential and ATP syntheses, thus inducing a drop in ATP levels essential for cell function (Su et al., 2009; Lapresta-Fernàndez et al., 2012). At the same time, the strong respiratory activity observed following the exposure of the isolated roots to SA could be explained by the entry into activity in the mitochondria of the second mitochondrial respiratory pathway (Alternative pathway), which is often triggered under stressful situations in plants (Djebar and Djebar, 2002; Ali et al., 2009).

5 Conclusion

In conclusion, this work showed new data on the risks of drugs and their secondary products on non-target organisms such as plants. However, it seems that the treatment with PAR and SA affects the behavior of isolated bean roots in different ways; since Par is likely to cause inhibiting the metabolic processes, while SA roots are mostly to have a positive effect on these processes. This is probably owing to the fact that SA is a compound that is synthesized by the plant itself.

Ethical statement

All applicable international, national, and/or institutional guidelines for the care and use of biological materials were followed.

Conflict of interests

The authors declare that there are no conflict of interest

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