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Original Research Article

Growth and Total Phenolic Contents of *Arthrospira platensis* (Nordstedt) Gomont in Response to Salicylic Acid[*]

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ABSTRACT

In this study, the effects of salicylic acid on growth of *Arthrospira platensis* (Nordstedt) Gomont were investigated. For this reason, different concentrations of salicylic acid (0.5, 1, 1.5, 2 g L^{-1}) were given to cultures, then changes of optical density, chlorophyll-a and total phenolic contents were assessed. OD and methanol extract of chlorophyll-a and phenolic contents were measured by spectrophotometrically. All parameters decreased with increasing salicylic acid concentrations and LC_{50} value was 1.5 mg L^{-1} for ninth day. Growth of *A. platensis* declined with salicylic acid treatment.

Key Words: *Arthrospira platensis*, biomass, chlorophyll-a, phytohormones, salicylic acid, total phenolic contents

Abbreviations: 6-BA: 6-Benzyl Adenine, GA3: gibberellin, IAA: indole asetic acid, $LC_{50:}$ median lethal concentration, α-NAA: α-naphthylacetic acid, OD: Optical Density, TA: Triacontanol, USA: United States of America

Salisilik Asitin, Arthrospira platensis (Nordstedt) Gomont Alginin Gelişimi ve Toplam Fenolik İçeriği Üzerine Etkisi

ÖZET

Bu çalışmada salisilik asitin, *Arthrospira platensis* (Nordstedt) Gomont alginin gelişimi üzerine etkisi araştırılmıştır. Salisilik asitin farklı konsantrasyonları (0.5, 1, 1.5, 2 g L-1) alg kültürüne verilerek optik yoğunlukta (OD), klorofil-a konsantrasyonunda ve toplam fenolik içerikte meydana gelen değişiklikler ölçülmüştür. OD, klorofil-a ve fenolik içerik spektrofotometrik olarak belirlenmiştir. Artan salisilik asit konsantrasyonuna bağlı olarak tüm parametrelerde azalma görülmüştür ve deneyin dokzuncu gününde LC_{50} değeri 1.5 mg L^{-1} olarak belirlenmiştir. Sonuç olarak salisilik asit uygulamasının *A. platensis* alginin gelişimini azalttığı görülmektedir.

Anahtar Kelimeler: *Arthrospira platensis*, Biokütle, Klorofil-a, Fitohormonlar, Salisilik asit, Toplam fenolik içerik

INTRODUCTION

The unrestricted developmental activities carried out during the last decade have given rise to serious environmental problems, and a general increase in the level of pollutants poses serious problems for the natural ecosystem (Coudhary et al. 2007). Algae are used in large-scale both in ecosystem monitoring studies and water eco-toxicology studies that conducted to determine the maximum tolerated concentrations of pollutants. Numerous studies have been performed about the effects of environmental pollutants, especially heavy metals, on the development of algae (Pempkowiak and Kosakowska 1998, Shehata *et al.* 1999, Wu *et al.* 2008, Elbaz *et al.* 2010). In these studies, *Arthrospira platensis* (Nordstedt) Gomont has also been used as a model organism to determine the effect of the pollution (Şeker *et al.* 2008; Murugesan *et al.* 2008, Fang *et al.* 2011, Çelekli and Bozkurt 2011).

Salicylic acid is an intrinsic growth regulator that is synthesized from an aromatic ring with a hydroxyl group by plants or some microorganisms (Klessig and Malam 1994, Pieterse and Loon 1999, Shah 2003). The signaling molecules can inhibit the ethylene biosynthesis, root adsorption and membrane mechanism (Khan *et al.* 2003, Singh and Usha 2003). Anthocyanin production, adventive rooting and nastic leaf movements are stimulated with its' treatment (Sudha and Ravishankar 2003).

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Only a few studies have shown the influence of vegetable hormones on algae, and these studies have primarily focused on the beneficial effects of these hormones. However, generally their environmental hazards have been ignored (Pan *et al.* 2008). Salicylic acid is an important signaling molecule involved in both local defense reactions at infection sites and the induction of systemic resistance, so it is also used for medical purposes (Senaratna 2000, Shah 2003, Loake and Grant 2007). Kshirsagar (2010) found that biomass of *Arthrospira* increased as a result of the pharmaceutical industry wastewaters. In this study, we aimed to test whether Salicylic acid has an effect on the growth and total phenolic contents of *A. platensis*. We wished to evaluate the effects of this hormone on aquatic ecosystems which are contaminated with the medical waste waters.

MATERIALS AND METHODS

Spirulina (*Arthrospira*) *platensis* M2 (SLYSP01) was provided from Soley Microalgae Institute (California, USA). It was cultured in Spirulina Medium (Aiba and Ogawa 1977) with non axenic conditions. 20 mL algae culture was inoculated to 180 mL culture medium in 250 mL Erlenmeyer flask and was incubated at 30ºC, with 5000 lux photon dense (12 hours light, 12 hours dark) in growth chamber on ten days. Afterwards, *A. platensis* cultures were refreshed again for the treatment of salicylic acid. Different concentrations of salicylic acid (0.5, 1, 1.5, 2 g L^{-1}) was added to *A. platensis* cultures. For the control experiment, *A. platensis* biomass was grown under identical culture conditions without adding any salicylic acid. Treatments were made with three replicate and cultures was incubated for nine days. OD of each *A. platensis* culture was measured at 560 nm wavelength with spectrophotometric methods with two days interval during the study period. The absorbance was plotted to dry weight with equation:

$CDW=0,672*OD_{560}+0.028$ (Xue *et al.* 2011)

Chlorophyll-a was measured according to Youngman (1978) and total phenolic contents were measured with Folin-Ciocalteu method according to Singleton and Rossi (1965) at the end of the ninth day.

OD values were evaulated with lineer regression, while chlorophyll-a and total phenolic contents were evaulated with logaritmic regression (Microsoft, 2003).

RESULTS AND DISCUSSION

Effects of salicylic acid on biomass production of *A. platensis* were evaluated with absorbance at OD 560 nm. Exogenous addition of different concentrations (0.5, 1, 1.5, 2 g L^{-1}) of salicylic acid showed varying toxicity to *A. platensis.* The biomass values decreased with increasing concentration of the salicylic acid (Figure 1) and LC_{50} value was 1.5 mg L^{-1} at the end of the ninth day (r=0.9242) (Figure 2).

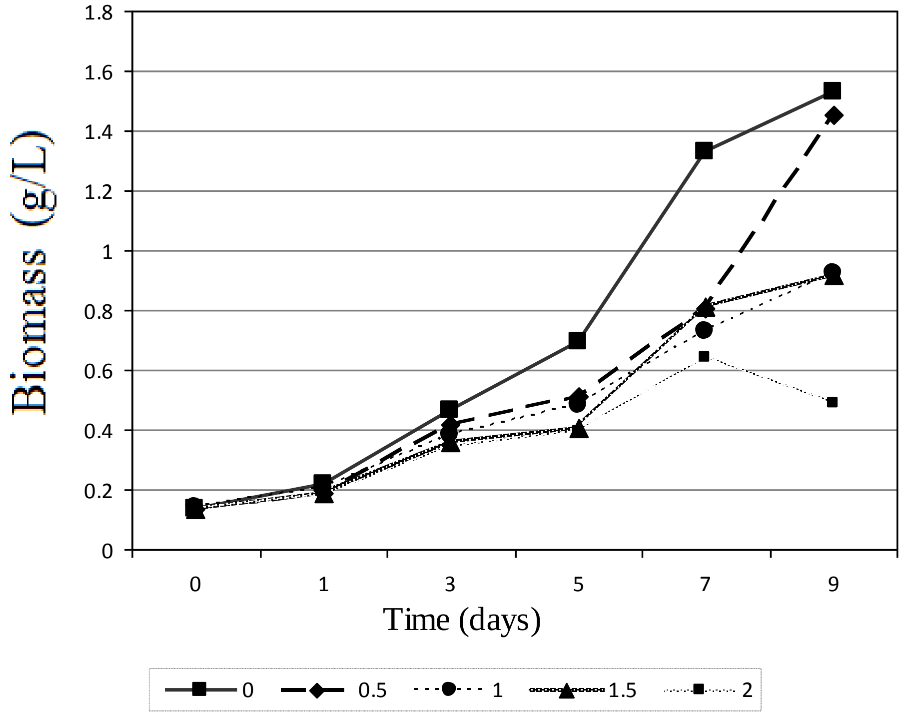

Figure 1. Changes in biomass values (g L^{-1}) of *A. platensis* with cultivation time under different concentrations (0.5, 1, 1.5, 2 g L^{-1}) of salicylic acid.

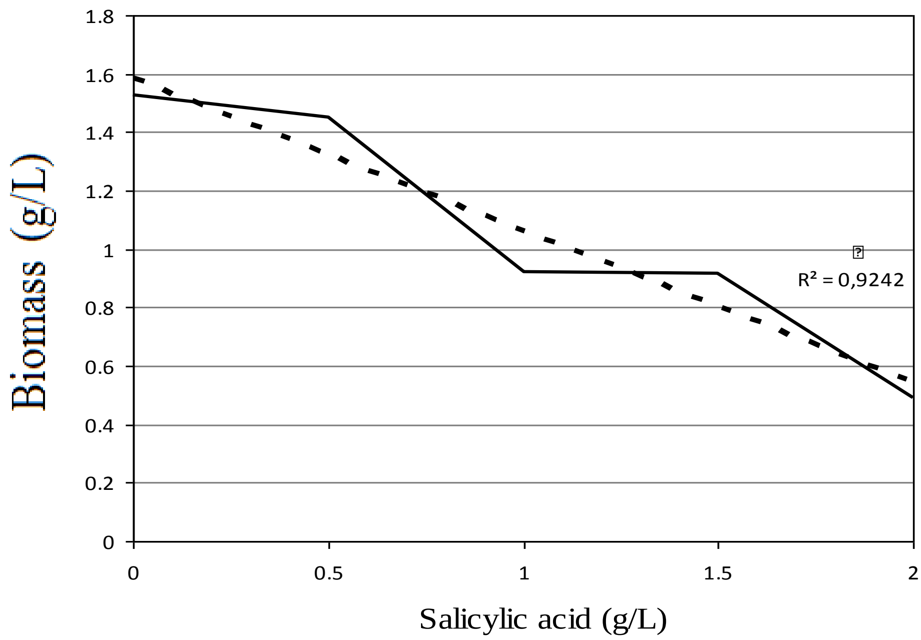

Figure 2. Changes in biomass values (g L^{-1}) of *A. platensis* under different concentrations (0.5, 1, 1.5, 2 g L^{-1}) of salicylic acid at the end of the ninth day.

Phytohormones have different effects on algae. Some plants' growth hormones increased the growth of Cyanobacteria. Triacontanol (TA), gibberellin (GA3) and indole asetic acid (IAA) advanced the growth of *Pavlova viridis* (Yang *et al.* 2000). Li *et al.* (2002) reported the influences of different concentrations of triacontanol (TA), gibberellin (GA3) and α-naphthylacetic acid (α-NAA) on the growth of *Amphora coffeaeformis* and *Navicula corymbosa*. GA3 stimulated the growth of these algae at an optimal concentration of 0.5 mg L^{-1}, while TA and α-NAA both stimulated the growth at an optimal concentration of 1.0 mg L^{-1}. Shi *et al.* (2004) studied the influence of six plant growth regulators on the growth of *A. platensis* A (9), and they found 0.5 mg L^{-1} GA3, 1.0 mg L^{-1} 6-Benzyl Adenine (6-BA) and 5 mg L^{-1} NAA had significant effects on the growth of strain *A. platensis* A(9). Moreover, Pan *et al.* (2008) showed that GA3 promoted the growth of *Microcystis aeruginosa* at an optimal concentration of 25 mg L^{-1}.

On the other hand, other studies have shown that plants' hormones had inhibitory effect on growth of Cyanobacteria (Bendana and Fried 1967, Evans and Sorokin 1971). Leganes *et al.* (1987) investigated the effect of IAA on the growth of *Anabaena* PCC 7119 and *Nodularia* sp. Concentrations higher than 10^{-4} M and 10^{-5}M of IAA were inhibitory effect on the growth of *Anabaena* PCC 7119 and *Nodularia* sp., respectively. Park *et al.* (2006) reported that *Microcytis* growth was significantly inhibited with the addition of salicylic acid. Our results showed similar results that salicylic acid inhibited to growth of *A. platensis*.

Effect of salicylic acid treatment on the variation of chlorophyll-a and total phenolic contents of *A. platensis*, at the end of the ninth day, were shown in Figure 3 and 4. Chlorophyll-a and total phenolic contents of *A. platensis* decreased with increasing concentration of the salicylic acid (r=0.9082, r=0.7412, respectively). Measuring the concentration of pigments for evaluating the impact of any chemicals on algae, is both easy and suitable for measuring the stress, and chlorophyll-a was the most commonly measured pigment in algae (Rai *et al.* 1992). Pan *et al.* (2008) showed that 10 and 25 mg L^{-1} treatment of GA3 on *M. aeruginosa* enhanced chlorophyll-a values. Moreover, Hunt *et al.* (2011) reported that naphthalene acetic acid, solubilized in ethanol, increased chlorophyll-a values and stimulated the growth of diverse species of microalgae. Phenolic compounds such as flavonoids, phenolic acids, and tannins which possess diverse biological activities, such as anti-inflammatory, anti-atherosclerotic and anti-carcinogenic activities, are considered to be major contributors to the antioxidant capacity of plants (Li *et al.* 2006, Chung *et al.* 1998). The environmental control of phenolic production are essential in optimizing the application and reliability of algal biomonitoring (Connan and Stengel, 2011). However, only a few studies have performed on variability of algal phenolic contents under different stress conditions (Ragan and Glombitza 1986, Ragan and Jensen 1978, Connan and Stengel 2011). In these studies, algal phenolic contents were induced by changes in irradiance, temperature, salinity and increased copper concentration. These results were contrary to our findings. Only one study was performed on *Arthrospira* species and in this study, the total phenolic contents of *Spirulina* (*Arthrospira*) *maxima* were 15.4 mg total phenolics in 1 g of dry algae matter, however they did not determine the variations of phenolic contents under different stress conditions (Miranda *et al.* 1998).

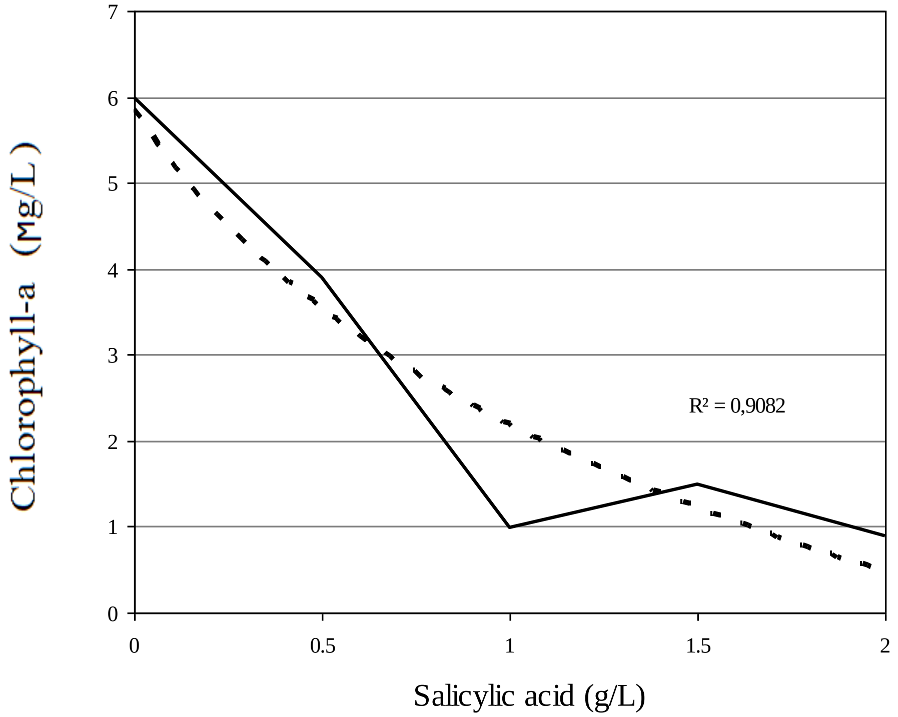

Figure 3. Changes in Chlorophyll-a values (µg L^{-1}) of *A. platensis* under different concentrations (0.5, 1, 1.5, 2 g L^{-1}) of salicylic acid at the end of the ninth day.

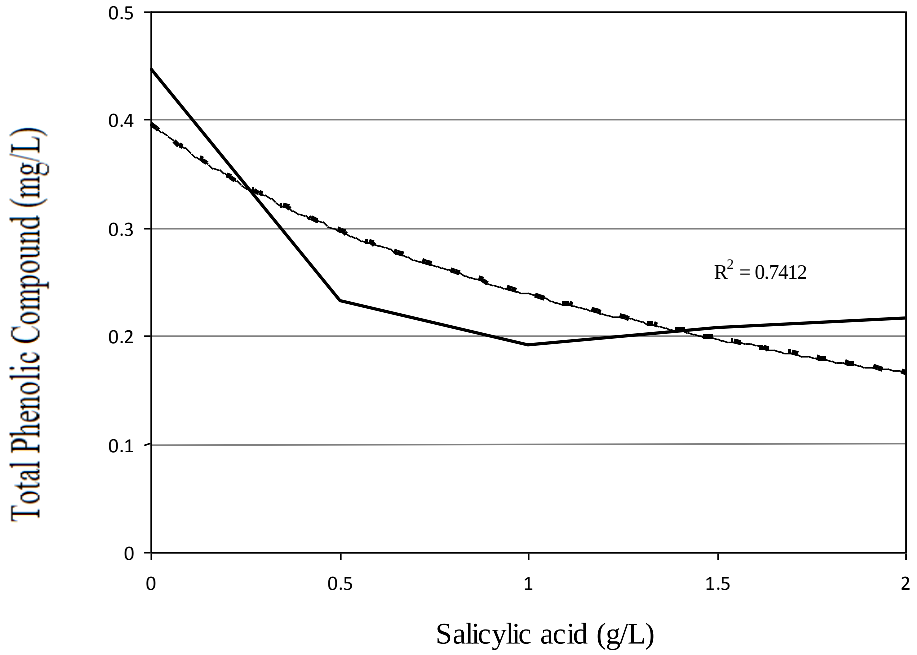

Figure 4. Changes in Total Phenolic Compounds (mg L^{-1}) of *A. platensis* under different concentrations (0.5, 1, 1.5, 2 g L^{-1}) of salicylic acid at the end of the ninth day.

CONCLUSIONS

In conclusion, Salicylic acid has a negative effect on the growth of and total phenolic contents of *A. platensis.* Waters contaminated with medical waste including Salicylic acid may effect the algal composition and the ecosystem. For this reason, detrimental concentrations of salicylic acid in aquatic systems should be determined and decreased in terms of environmental protection. Further studies are also required to elucidate the mechanism by which Salicylic acid decreases the growth and total phenolic contents of *A. platensis* at a molecular level.

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