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Quantitative Analysis of Absorption of APAP and ASA by *Oryza sativa L.* Plants under Variable pH Conditions

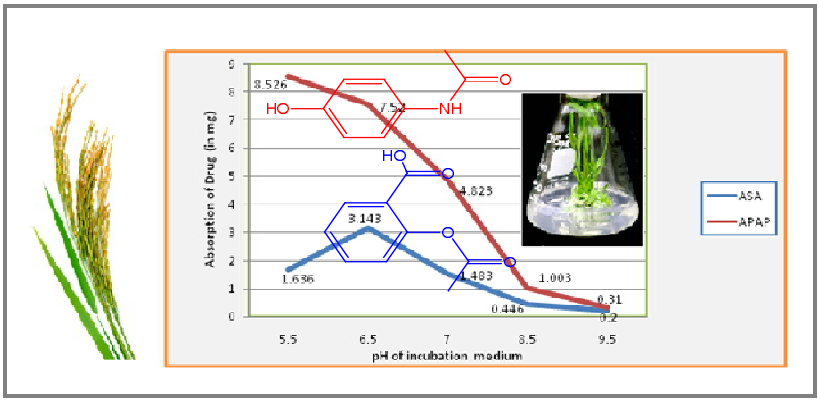
K. Abhijit*, G. Sirisha, M. Prasanna, Ayesha Nazneen and S. Sushma

Department of Chemistry, Tara Govt. (Autonomous) College,
Sangareddy-502001, Telangana, **INDIA**
Email: abhichem2010@gmail.com

ABSTRACT

Plants do absorb organic compounds efficiently compared to metal ions or other ionic compounds but if the organic compounds quite soluble in water and having active polar functional groups plants can absorbs organic molecules for some extent. Usually amino acids and sugars are absorbed by root cells of plants by co transport with H^+. But the exact mechanism was still not revealed. PAOMs like drugs having reactive polar functional groups like –OH, -COOH, -NH_2 etc. are considerably absorbed by plant's root system. The functional groups of drugs bind with proteins of plasma membranes of root cells and initially get accumulated in roots, from there the accumulated drugs distributed to different parts of plants through phloem due to Osmotic and Pressure gradients. Absorption of APAP and ASA by Oryza sativa L. plants were greatly affected by pH conditions. The optimal pH for maximum absorption was observed at 6.5 for ASA and 5.5 for APAP by oryza sativa L. plants. The quantity of APAP absorbed by 11th leaf Oryza sativa L. plant incubated in medium having pH around 5.5 was enhanced by 24.687 % compared to plants incubated in neutral medium (pH=7), whereas the increase was only 1.103 %, in the case of absorption of ASA by plants under similar conditions. But at the optimal pH point for ASA absorption i.e. at 6.5 the increase in absorption was 11.067 % compare to plants incubated in neutral conditions.

Graphical Abstract



Absorption of APAP and ASA by *Oryza Sativa L.* plants

Keywords: Acetyl-para-aminophenol, Acetyl salicylic acid, Para Amino Phenol, Physiologically Active Organic Molecules, Salicylic Acid.

INTRODUCTION

Plants do absorb organic compounds efficiently compared to metal ions or other ionic compounds but if the organic compounds quite soluble in water and having active polar functional groups plants can absorbs organic molecules for some extent. Usually amino acids and sugars are absorbed by root cells of plants by contransport with H^+ [1-3]. But the exact mechanism was still not revealed. Physiologically active organic molecules (PAOMs) like drugs having reactive polar functional groups like –OH, -COOH, $-NH_2$ etc. are considerably absorbed by plant's root system. The functional groups of drugs bind with proteins of plasma membranes of root cells and initially get accumulated in roots, from there the accumulated drugs distributed to different parts of plants through phloem due to Osmotic and Pressure gradients. The current paper deals with estimation of quantitative absorption of Acetyl salicylic acid (ASA) and Acetyl para amino phenol (APAP) through aqueous solution by paddy plants under different pH conditions.

Due to good amount of solubility of ASA and APAP in water, these drugs were administered to the plants through aqueous solutions. Plants absorb fair amount of solutes which dissolves in water. Quantitative measurement of Absorption of ASA and APAP by plants possibly determined when the drug should be stable under experimental conditions. To minimize the decomposition of drug during absorption, the experiment was conducted for short duration (24 h). It is very difficult to analyze the exact quantity of drug absorbed by plant, because of absorbed drug would degraded or transformed into various other compounds. To overcome this problem quantity of drug absorbed by plants can be correlated with the drug displaced from culture drug solution in which plants were incubated. The drug displaced is always proportional/equal to the drug absorbed by the plants.

MATERIALS AND METHODS

Plant material and exposure: Experiments were performed with *Oryza sativa L.* using hydroponic incubation under different photo (Light), pH, time duration and biomass variations. Paddy plants were collected from agricultural fields of sangareddy area. The paddy plants with 11^{th} leaf growth were selected for experiment. These plants were sterilized with 0.2% w/v mercuric chloride solution and then washed with deionized water. Pure sample of APAP obtained from SD fine chemical ltd. India, was used for chemical treatment to plants and ASA was synthesized in the lab, was used for treating the plants. The each plant was incubated in 5 ml of 3 mg mL^{-1} aqueous solution of APAP and ASA in test tubes. The experimental design was conducted as factorial randomized set with three replications. Observation were made after 24 h of incubation and focused on quantity of drug displaced from aqueous medium. Test tubes were properly plugged with cotton plugs, after air was evacuated using vacuum pump and black paper wrapped to minimize the photo-oxidation and decomposition of the drugs.

Preparation of sample for Drug Estimation: Plant's root part (submerged parts) and test tubes (in which plants were incubated) were thoroughly washed with 25 mL of de ionized water (5 mL x 5 times) and collected residual sample of drugs remained.

Preparation of APAP Solution for Estimation [4, 5]: The residual APAP sample solution collected from plants was taken in 50 mL RB flask and refluxed with 2 mL of 4M HCl for about 30 min. The content was diluted and make up 50 mL solution in 50 mL volumetric flask. 1 mL of this solution taken into 50 mL volumetric flask and to this aliquot 0.6 mL of 4M HCl and 1 mL of 0.1 w/v solution sodium Nitrite were added for diazotization. One ml of 0.5 % w/v solution of ammonium sulphamate was added after 3.5 min to destroy excess HNO_2 and left for 3 min. To this content, 1.5 mL of 0.5% w/v solution of 1-Napthol in 4M NaOH was added as coupling agent. The azodye developed was diluted upto the mark (50 mL). Same procedure repeated for all the samples collected from plants. Absorbance was measured at 505 nm wave length using Systronics 106 automat spectrophotometer and APAP was estimated from calibration curve.

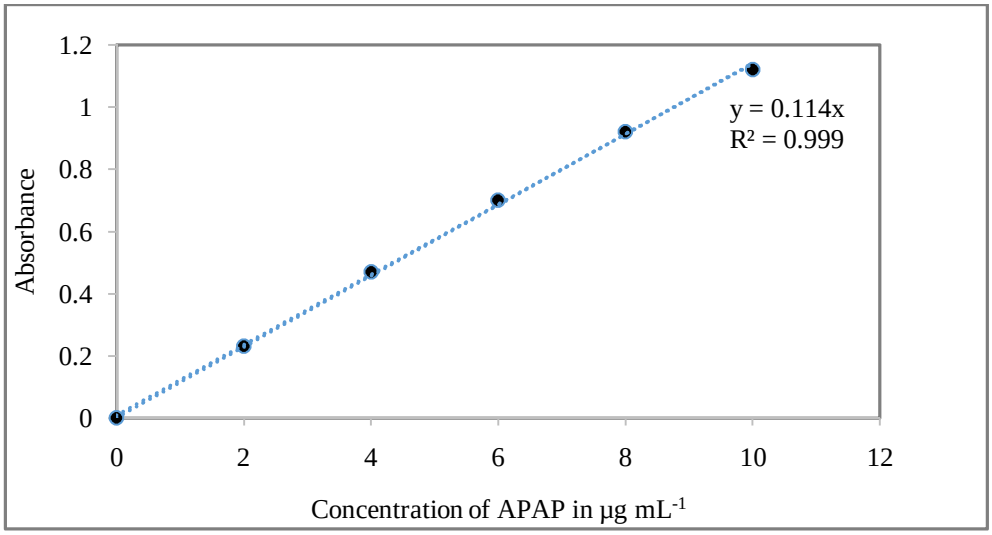


Figure 1. Calibration graph of standard APAP solution using UV-Vis Spectroscopy.

Preparation of ASA Solution for Estimation [6-10]: The residual ASA sample solution collected from plant's culture medium after 24 h of incubation was taken in 50 mL RB flask. To this solution 1 ml of 0.5M NaOH solution was added and heated to boiling. The solution was cooled , transferred quantitatively to a 250 volumetric flask and diluted to 250 mL by adding deionized water and 1 mL of 0.02 M $Fecl_3$ solution by maintaining acidic conditions (pH range 2.6 to 2.8) using 0.002M HCl solution. Same procedure repeated for all the samples collected from plants. The absorbance of colored solution at 519 nm (λ_{max}) using Systronics 106 automat spectrophotometer and ASA was estimated from calibration curve.

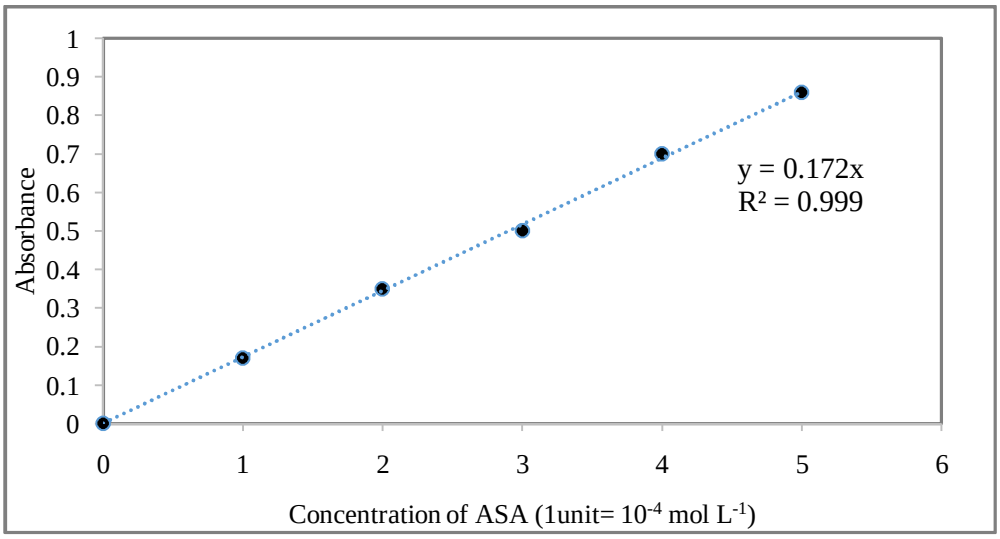


Figure 2. Calibration graph of standard ASA solution using UV-Vis Spectroscopy.

Spectrophotometric Estimation of ASA and APAP: Pure sample of APAP obtained from SD fine chemical ltd. India, was used for chemical treatment to plants and the same sample of APAP was used as standard. Accurately 250 mg of pure APAP was weighed out and refluxed with 20 mL of 4M HCl with 30 mL of distilled water for about 30 min to prepare a standard solution. The content was appropriately diluted and required aliquots were taken for plotting of calibration curve.

Solution containing 2- 10 µg mL^{-1} of APAP equivalent was taken in 25 mL volumetric flask. To this aliquot 0.6 mL of 4M HCl and 1 mL of 0.1% w/v solution of sodium Nitrite were added for diazotization. One mL of 0.5 % w/v solution of ammonium sulphamate was added after 3 min to decompose excess HNO_2 and left for 2 min. Then after 1.5 mL of 0.5% w/v solution of 1-Napthol in 4M NaOH was added as coupling agent. The absorbance of the azodye was measured at 505 nm.

Similarly, Pure form of ASA synthesized in lab and purity was checked by using HPLC. This sample of ASA was used as standard and same ASA was used for chemical treatment to plants.

To prepared 400 mg of prepared ASA, 10 mL of 1M NaOH solution was added and the solution heated to boiling. The solution was cooled, transferred quantitatively to a 250 mL volumetric flask and diluted upto the mark with distilled/deionized water. This solution was labelled as "standard ASA". Using the standard ASA, a series of aliquots ($1x10^{-4}$ to $5x10^{-4}$) were prepared by taking different volumes of standard ASA, and diluting to 100 mL by adding equivalent amount of 0.02M $FeCl_3$ solution [**10**]. These aliquots were taken for preparation of calibration curve.

RESULTS AND DISCUSSION

Absorption of APAP and ASA by *Oryza sativa L.* plants were greatly affected by pH conditions. The optimal point for maximum absorption was observed at 6.5 for ASA and 5.5 for APAP by *oryza sativa L.* plants (Table 1). The quantity of APAP absorbed by 11^{th} leaf *Oryza sativa L.* plant incubated in medium having pH around 5.5 was enhanced by 24.687 % compared to plants incubated in neutral medium (pH=7), whereas the increase was only 1.103 %, in the case of absorption of ASA by plants under similar conditions. But at the optimal pH point for ASA absorption i.e. at 6.5 the increase in absorption was 11.067 % compare to plants incubated in neutral conditions.

Table 1. Absorption of ASA and APAP by 11^{th} leaf paddy plant after 24 h of Drug incubation at different pH Levels.

S.No.	pH levels (Approximately)	Drug Treatment	Concentration of Absorbed Drug (in mg)	Percentage of Absorption
1	7	ASA	1.483±0.308	9.886
2	5.5	ASA	1.636±0.126	10.906
3	6.5	ASA	3.143±0.835	20.953
4	8.5	ASA	0.446±0.069	2.973
5	9.5	ASA	0.200±0.015	1.334
6	7	APAP	4.823±0.262	32.153
7	5.5	APAP	8.526±0.866	56.840
8	6.5	APAP	7.520±0.866	50.133
9	8.5	APAP	1.003±0.024	6.686
10	9.5	APAP	0.310±0.109	2.067

Values are mean ± S.D. (n=3)

On the other hand absorption of APAP and ASA drastically decrease under basic conditions. The absorption of APAP by *oryza sativa L.* was only around 6.686% at pH 8.5, furthermore the absorption decreases to 2.066 % at pH 9.5, whereas absorption of ASA was 2.973% at pH 8.5 and 1.334% at 9.5 pH under similar conditions (Figure 3).

In fact, APAP and ASA were hydrolyzed conveniently under basic and acidic conditions. APAP has amide linkage whereas ASA has Ester linkage; both of these linkages were hydrolyzed either by acid or a base. The hydrolysis products salicylic acid (SA), from ASA and para amino phenol (PAP), from APAP are more reactive than APAP and ASA as they have more reactive functional groups ($-NH_2$, OH). These reactive products were easily absorbed by plants under acidic conditions.

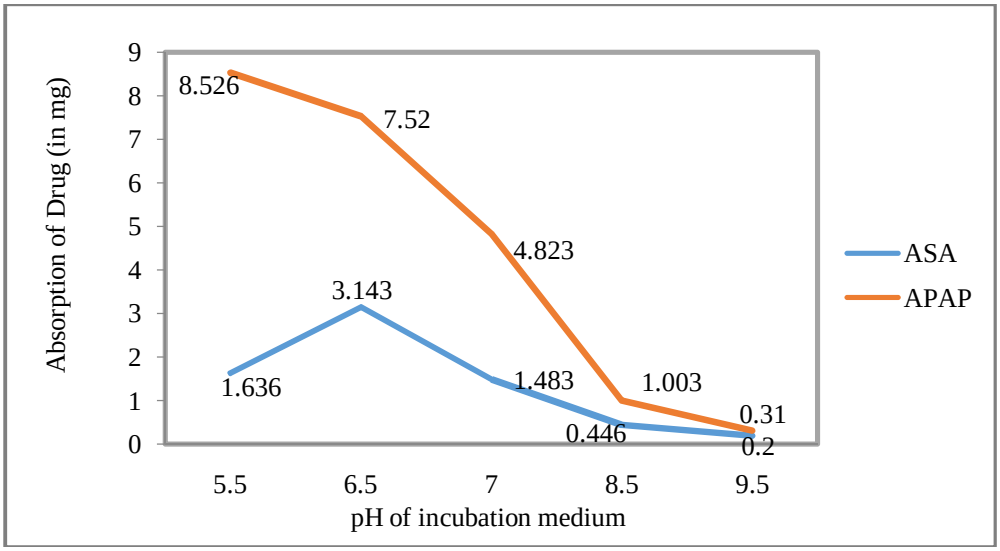


Figure 3. Impact of pH of incubation medium on drug absorption by paddy plant.

In this context, the absorption of APAP and ASA was increased in acidic conditions due to several factors. The major factor is loosening cell walls (increase in plasticity) of root cells due to H^+ ions which increase permeability of the root cells. Presumably loosening takes place because the H^+ ions activates several enzymes that degrades rigid polysaccharides within the walls [**11-15**]. Transport of organic molecules move into root cells largely by co-transport with H^+ ions just like absorption of amino acids and sugars by root cells **[1-3]**.

Even high concentration of H^+ ions retards absorption of organic compound like drug which was clearly indicated by decrease in absorption of ASA by *oryza sativa L.* plants at pH 5.5 compared to pH 6.5. The decrease was around 10.047 % in *Oryza sativa L.* plants compared to absorption at pH 6.5. But the absorption of APAP was not retarded under higher H^+ ion concentration instead its absorption was increased by 6.706 % in *Oryza sativa L.* plants at pH 5.5, compared to absorption at 6.5. This is due to nullifying excess H^+ ions by PAP, (the hydrolyzed product of APAP) in the formation PAP H^+ ions, furthermore which is more easily permeable into root system due to its cationic nature.

APPLICATION

The outcome of the current project is providing strategic design for Phyto-remediation of certain organic pollutants by modulation of pH. It is evident from the study that phyto-absorption significantly depends on the pH conditions as H^+ ion concentration can affect the plant's biochemical system which leads to variations in many physiological parameters. The knowledge of the present project further applicable in the field of Phyto-remediation, Nutrient transportation and assessing the impact of chemical stress on plant's physiology.

CONCLUSION

In order to evaluate the quantitative absorption of APAP and ASA by *Oryza sativa L.* plants incubated by *in vitro* method under different pH conditions, quantity of APAP and ASA displaced from culture medium was estimated by using spectrophotometry method. The results showed that the optimum pH determined for absorption of APAP was 5.5 and for ASA it was 6.5, whereas, the basic conditions in the hydroponic incubation medium greatly inhibited the absorption. It is evident from the study that absorption of these drugs was maximum in acidic pH conditions under limited period of exposure.

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