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Impact of Commercial Detergents on Organogenesis in Allium Cepa L and Utility of Foldscpe Tool

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Abstract

Detergents are chemically made cleaning agents extensively used in households, industries; hospitals etc. and the spent detergents are released into nearby water bodies. Such detergent polluted water is often utilized for agriculture and home garden irrigation purposes. Much study has not been done to explore the impact of this polluted water on crop growth and its productivity. The current study tried to demonstrate the impact of some commonly used commercial detergents on crop growth by taking organogenesis in onion (*Allium cepa* L.) as model system and by utilizing low cost, origami based paper microscope tool i.e. foldscope. Our study divulged that for most of the detergents the minimum inhibitory concentration of organogenesis was 0.5% and compromised organogenesis was observed at low detergent concentrations (0.006, 0.012 and 0.025%). Stunted roots and decreased root density in onions were observed at higher detergent concentrations. Altered cell morphology (more dehydrated cells) and disordered cellular arrangement was observed in roots that were grown in high detergent concentrations by using Foldscope.

Keywords: *Allium Cepa* L., Commercial Detergents, Organogenesis, Origami, Foldscope

Introduction

Detergents are cleaning agents, made up of a cocktail of chemical components and are available as powders or concentrated solutions (Smulders E et.al; 2009). Generally they are sodium salts of long chain alkyl hydrogen sulphate or a long chain of benzene sulphonic acid (The "Gold Book"; 1997). Detergents are a set of compounds with amphiphilic structures, where each molecule has a hydrophilic polar head and a long hydrophobic non-polar tail. The hydrophobic portion of these molecules may be straight or branched chain of hydrocarbons or it may have a steroid structure. The hydrophilic portion is more diverse, they may be ionic or non-ionic, and can range from a simple or a relatively elaborate structure (Neugebauer and Judith M. 1990). Due to its amphiphilic and

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surfactant nature detergents are extensively used in home cleaning and industrial processes (Effendi I et.al; 2017). Moreover, detergents work better at basic or alkaline pH (Niir Board; 1999). The spent detergents and surfactants released from home and industry enter in to the water bodies causing far-reaching environmental impacts (London A.S et.al; 2016). Detergents affect the fauna and flora, and they have direct and indirect effects on ecosystems. Eutrophication, foaming, and altering parameters such as temperature, salinity, turbidity and pH are more important and their effects need to be assessed, managed and controlled (Seyyed Alireza Mousavi and Farank Khodadoost; 2019). Few reports showed that more than 95% of the commercial detergents can cause damage or disorders to the respiratory tract, reproductive system, endocrine system and immune systems (Dingle P et.al; 2002). However, no significant reports have been found on detergents effect on agriculture and its productivity. In this context to demonstrate the impact of detergents on crop growth and development we adopted low cost, affordable models: organogenesis (Roots and shoots development) in onions (*Allium cepa* L.) and foldscope tool. Foldscope is an origami based, affordable (~ $1 cost), simple microscope of 140X magnification that can be assembled from paper, lens and magnets (Lhanjey P.W et.al; 2019). The foldscope can connect with mobile phone, take photographs and videos of the specimen and also could project image on the screen. It was designed by Dr. Manu Prakash, an Indian born scientist at Stanford University-USA and aims to make inexpensive and easy tools available for scientific use in the world, especially in developing countries (Cybulski J.S; 2014). Currently the Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India is extensively popularizing this foldscope tool across the country through workshops and micro grant projects. Keeping these aspects in view, in the present study we planned to assess the influence of various commonly used detergents on root generation and shoot generation in *Allium cepa* L. Further we sketch to annexure the impact of detergents on root morphology using foldscope instrument and also to depict the foldscope potential to use it as an educational and research tool.

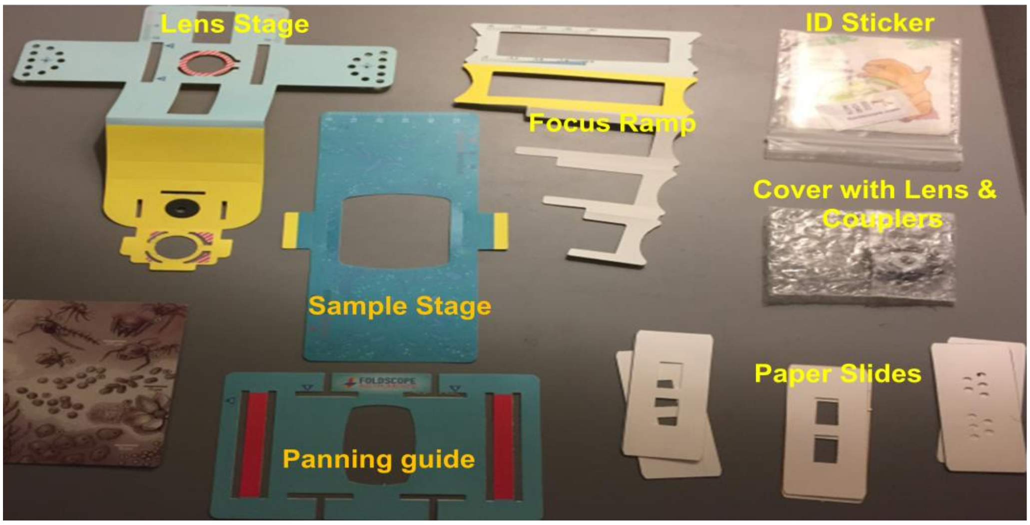
Materials and Methods

The foldscopes used in this study was offered by DBT-New Delhi under the Indo-US Foldscope project. The powder detergents (Rin, Ariel, Tide, Ghadi, Surfexel, Sunflower, Wheel, Urvashi & XXX) and Onions collected from local super market. Saffranin stain and other lab materials procured from National Scientific Products, Kakinada, A.P. All the experiments were conducted at Biotechnology Laboratory of Government Degree College (Men)-Srikakulam, A.P.

Assembly of Foldscope (AF)

Foldscope is a simple microscope made it from paper (Assembly sheet), magnets (3-4 no) and a lens (140x). The assembly sheet comprises of the following major parts- Lens Stage, Sample stage, Focus ramp and Panning guide (Lhanjey P.W et.al; 2019). The details of the assembly process made by our foldscope team given in this link: https://youtu.be/dgE8GeuLL4w and the photographs of various parts of foldscope shown in fig-1.

Fig-1: Various parts of Foldscope



Determination of Minimum Inhibition Concentration (MIC)

MIC is the minimum concentration of detergent in the solvent (water) greater than which inhibits the organogenesis in onions. In order to find the MIC, we prepared a series of detergent concentrations of 500 ml volume for the selected detergents starting from 10%, 5%, 2.5%, 1%, 0.5%, 0.1%, 0.05% & 0.01%. Each concentration in triplicates was used to place trimmed (old roots removed from bulbs) onions and subsequently determined the MIC after 20 days of incubation.

Organogenesis in Onions (OO)

Organogenesis is the process of generation of roots and green shoot primordium from the onions. After determination of MIC, to assess OO 1000 ml quantities of 0.1%, 0.05%, 0.025%, 0.012% and 0.006% solutions with Rin detergent powder were prepared. Then, 18 conical flasks of 250 ml volume were divided in to three triplicate groups each with six flasks. Each triplicate group was marked as 0.1%, 0.05%, 0.025%. 0.012%, 0.006% concentration and a control group. The control group of flasks was filled with tap water and the other flasks with the respective detergent solutions. Next 18 comparable onions were selected carefully dried roots were trimmed and placed on the top of the conical flask ensuring that the bulb was in contact with the solution for organogenesis as shown in fig-2. Similar procedure was followed for all the test detergents for organogenesis in onions.

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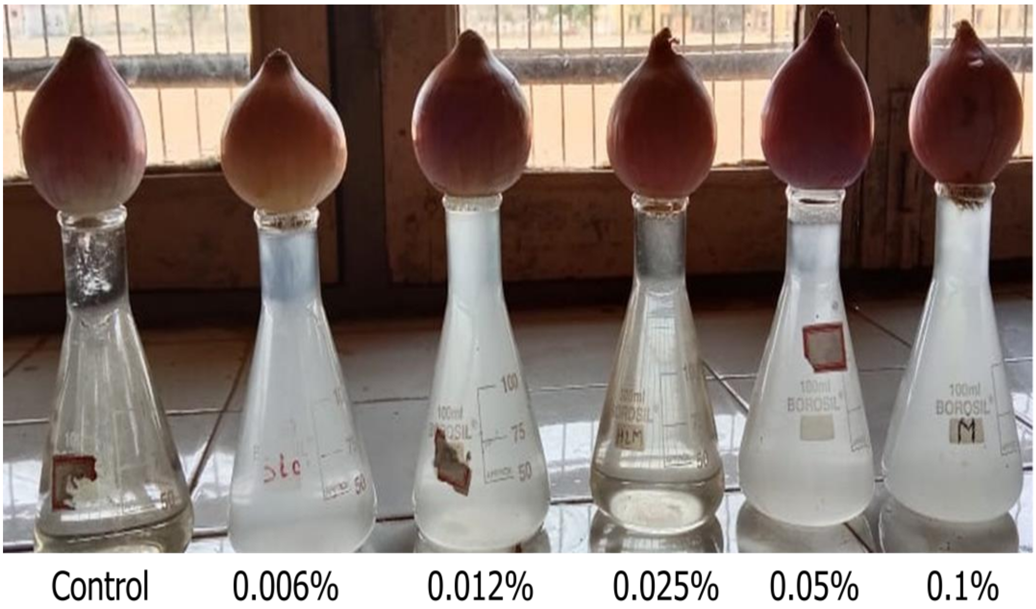
Fig-2: Conical flasks with various concentrations of Detergent



Assessment of Root and Shoot Density (ARSD)

ARSD is the process of determining the number of roots and shoots generated to the experimental onions. The following formula was used to measure the ASRD.

D = N ÷ A

D = Density; N = Number of Roots or Shoots; A = Area of Bulb or tip of the onion

$$A = \pi r^2$$

r = radius of the bulb or tip

$$\pi = \frac{22}{7} \text{ or } 3.14$$

ARSD was determined after 5 days, 10 days and 20 days of onion incubation. Every time the onions were taken out from the flask and carefully dried with tissue paper. Then the onions were placed on a clean contrast floor or paper shown in fig-3 and the roots and shoots of every onion under study counted manually. Also, the Area of every onion under study was determined by measuring radius and the density was calculated. The onions were then replaced in their respective flasks. Similar procedure was followed for all the test detergents and organ density calculated.

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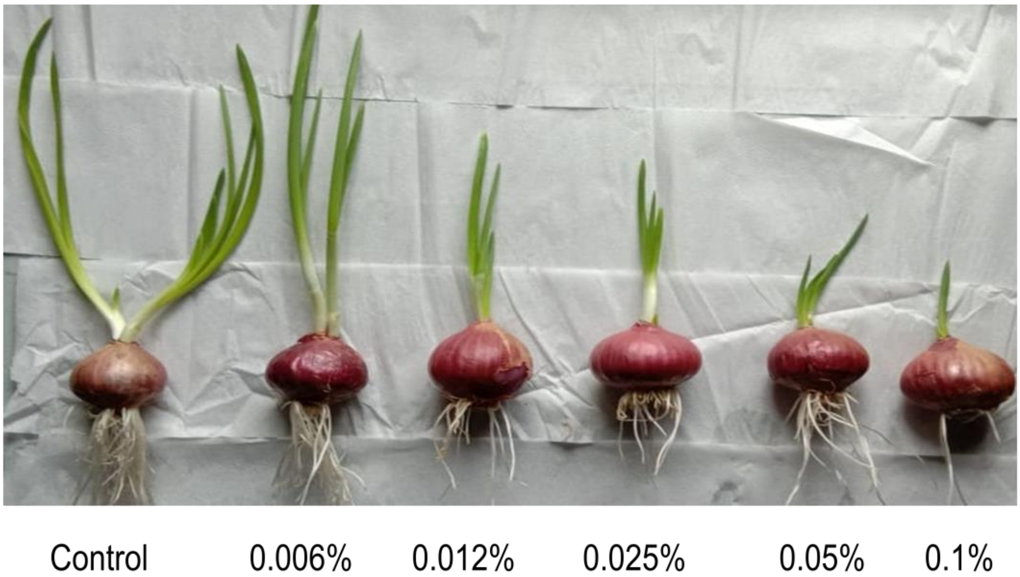
Fig-3: Organogenesis in onions after 20 Days of Incubation



Determination of Root and Shoot Length (DRSL)

DRSL was determined after 5 days, 10 days and 20 days of incubation. Every time the onions were taken out from the flask and carefully dried with tissue paper. The onions were then placed on a clean contrast floor or paper and the extreme lengths (shortest and longest) of root and shoot were measured using 30 cm scale. Then the average length was calculated using below formula

$A = (S + L) \div 2$

A = Average Length; S = Length of Shortest Root or Shoot; L = Length of Longest Root or Shoot

After study the onions were replaced in their respective plasks. Similar procedure followed for all the test detergents and the root & shoot lengths were calculated.

Study of Root Anatomy (SRA)

To study the influence of detergents on root anatomy, transverse sections (TS) of all the experimental onion roots were prepared, stained, mounted on slides and observed under foldscope. To do that the onion was removed from flask; the roots were dried carefully with blotting paper. Then a root was cut from the onion and held in between the index finger and thumb. Using sharp razor the root was sliced into thin sections, transferred into water containing watch glass using a brush. Later 2-4 good root TS were transferred into another watch glass with Saffranin stain and allowed to set for 5 minutes. Then the sections were transferred to the watch glass with water and set to rest for 2 minutes. One of the sections was placed in the middle of the slide. A drop of glycerin was added on the section and covered with a coverslip. Excess glycerin was wiped from the edges of the coverslip with a tissue paper and ensured that no air bubbles were formed in the mount. The mounted slide was inserted into the foldscope, adjusted and attached to the mobile and photographs of the sections were taken. Similar procedure was followed for all the other experimental onions.

Data Analysis

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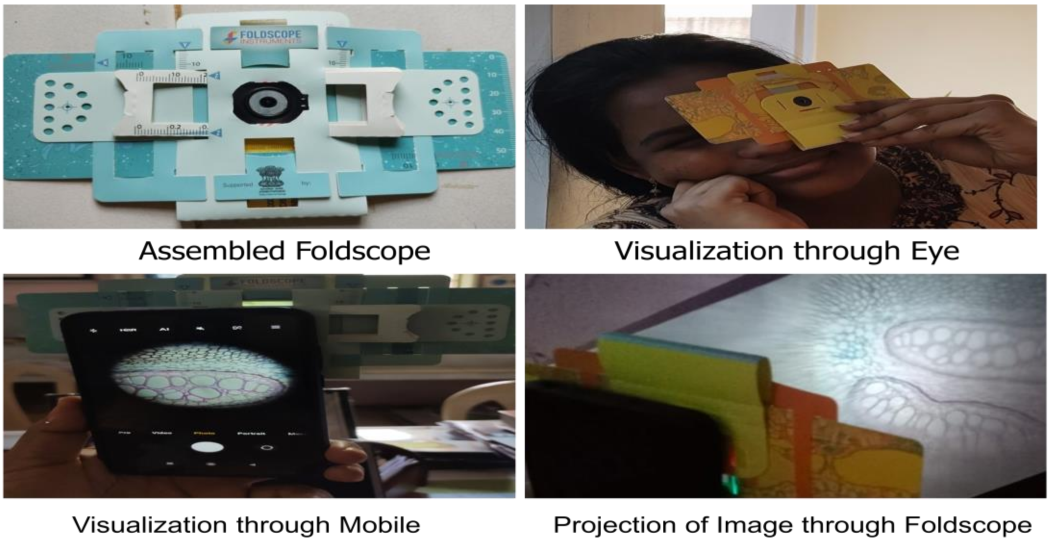
All the experiments were repeated independently in triplicates and all the calculations and graphs relative to control were done using Microsoft Excel 2007 software. Assembled foldscope and its usage methods shown in the fig-4.

Results

Fig-4: Different types of Foldscope Visualization Methods



The MIC for all the selected detergents was considered after 20 days of incubation. The MIC for Rin, Tide, Ghadi, Surfexel, Sunflower, Wheel, Urvashi detergents was 0.1% in water and for Ariel & XXX it was 0.5% and 0.05% respectively as shown in Table-1.

Table-1 Showing MIC for various detergents after 20 Days of incubation: X = No Organogenesis; Y = Organogenesis

Detergent Name **Concentration**	Rin	Ariel	Tide	Ghadi	Surfexel	Sunflower	Wheel	Urvashi	XXX
10%	x	x	x	x	x	x	x	x	x
5%	x	x	x	x	x	x	x	x	x
2.5%	x	x	x	x	x	x	x	x	x
1%	x	x	x	x	x	x	x	x	x
0.5%	x	Y	x	x	x	x	x	x	x
0.1%	Y	Y	Y	Y	Y	Y	Y	Y	x
0.05%	Y	Y	Y	Y	Y	Y	Y	Y	Y
0.01%	Y	Y	Y	Y	Y	Y	Y	Y	Y

Organogenesis in onions under all the testing detergents was done after 5 days, 10 days and 20 days of onion incubation. After 5 days of incubation roots development was observed only at 0.006%, 0.012% and 0.025% detergent groups along with control. In Ariel detergent roots were observed in control, 0.006%, 0.012%, 0.025% & 0.05%. However in XXX roots were found only in control, 0.006% & 0.012% concentrations. After 10 days of incubation roots were observed in all the concentration groups while green shoots were observed only in control and 0.006% groups. After 20 Days of incubation, roots and shoots were observed in all the groups with significant variations as shown in the fig-3.

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ARSD against time interval was calculated by using the given formula and average organ densities (Root densities only) for each detergent concentration and control shown in Table-2. Considerable improvement in the root density after 05, 10 and 20 days was observed only in control and at low detergent concentration group i.e. 0.006%. For 0.012%, 0.025% and 0.05% concentrations, notable improvement in root density was observed in between 05 to 10 days as shown in fig-5.

Table-2: Average root densities after 5, 10 and 20 days under each detergent concentration & control * Only Root Density Mentioned here for Simplification Purpose; D* = Days; Mode = The mode is the value that appears most often in a set of data values.

Concentration/ Detergent	ASRD in Control			ASRD in 0.006%			ASRD In 0.012%			ASRD in 0.025%			ASRD in 0.05%				ASRD in 0.1%	
	5D*	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**
Rin	4.7	14.4	21.1	4.1	12.9	16.9	3.6	9.1	10.4	2.7	7.4	8.7	--	5.3	7.4	--	0.7	0.9
Ariel	4.9	14.5	21.5	4.3	12.9	16.9	3.7	9.3	10.5	2.8	7.5	8.9	2.1	5.5	7.5	--	0.8	0.9
Tide	4.7	14.4	20.9	4.1	12.9	16.9	3.6	9.1	11.2	2.7	7.2	8.8	--	5.3	7.2	--	0.7	0.8
Ghadi	4.6	14.3	20.9	4.0	12.7	16.7	3.5	9.0	10.9	2.6	7.2	8.4	--	5.2	7.2	--	0.6	0.7
Surfexel	4.8	14.4	21.1	4.2	12.9	16.9	3.6	9.1	11.3	2.7	7.3	8.3	--	5.3	7.3	--	0.5	0.6
Sunflower	4.8	14.4	21.3	4.2	12.8	16.8	3.6	8.9	10.3	2.7	7.3	8.2	--	5.4	7.3	--	0.7	0.7
Wheel	4.7	14.3	21.1	4.1	12.5	16.5	3.6	9.1	10.3	2.7	7.3	8.3	--	5.3	7.3	--	0.6	0.8
Urvashi	4.9	14.5	22.0	4.3	12.8	16.8	3.7	9.3	10.2	2.8	7.2	8.6	--	5.2	7.2	--	0.5	0.7
XXX	4.5	14.2	20.3	3.8	12.4	16.2	3.4	8.9	9.9	--	6.9	7.9	--	5.1	6.9	--	0.4	0.5
Mode	**4.7**	**14.4**	**21.1**	**4.1**	**12.9**	**16.9**	**3.6**	**9.1**	**10.3**	**2.7**	**7.2**	**8.3**	**2.1**	**5.3**	**7.2**	**00**	**0.7**	**0.7**

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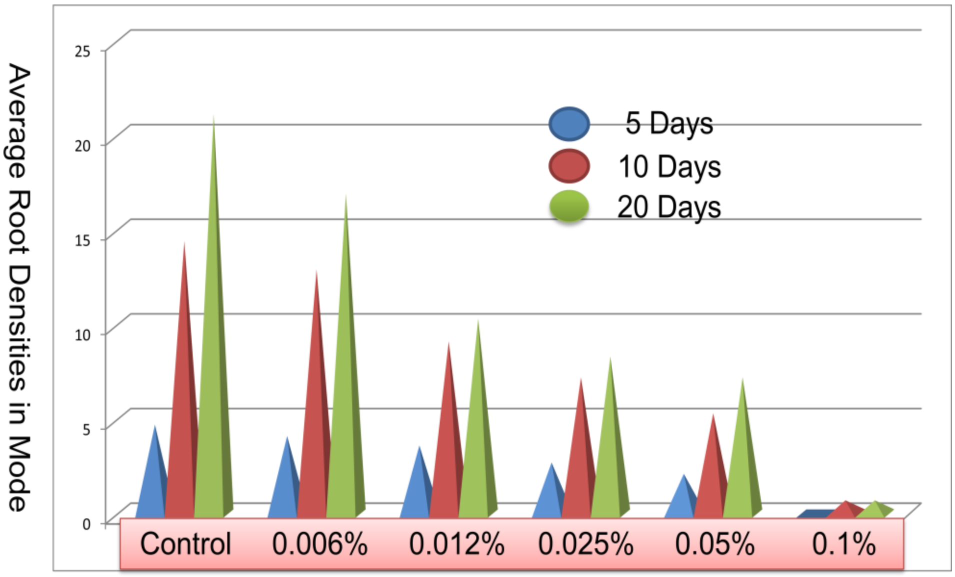
Fig-5: Root Densities over time intervals



DRSL against time interval was calculated as per given formula and average organ lengths (Root lengths only) for each detergent concentration & control shown in Table-3. Significant improvement in root lengths after 05, 10 and 20 days was observed in control and at low detergent concentration group i.e. 0.006% but no growth was observed after five days in 0.025% and 0.05% concentrations as shown in fig-6.

Table-3: Average root length after 5, 10 and 20 days under each detergent concentration & control

Concentration/ Detergent	DRSL in Control			DRSL in 0.006%			DRSL In 0.012%			DRSL in 0.025%			DRSL in 0.05%			DRSL in 0.1%		
	5D*	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**	**5D**	**10D**	**20D**
Rin	3.5 cm	7.5 cm	12.3 cm	3.4 cm	7.3 cm	12.2 cm	2.2 cm	5.5 cm	8.9 cm	1.9 cm	3.7 cm	5.7 cm	--	1.7 cm	3.3 cm	--	1.1 cm	2.1 cm
Ariel	3.6 cm	7.2 cm	12.4 cm	3.2 cm	7.1 cm	12.2 cm	2.6 cm	5.2 cm	8.9 cm	2.0 cm	3.6 cm	5.4 cm	1.3 cm	2.3 cm	3.8 cm	--	1.3 cm	2.1 cm
Tide	3.5 cm	7.4 cm	12.2 cm	3.5 cm	7.2 cm	12.1 cm	2.7 cm	5.6 cm	8.2 cm	1.7 cm	3.8 cm	5.8 cm	--	1.4 cm	3.0 cm	--	1.0 cm	2.0 cm
Ghadi	3.4 cm	7.2 cm	12.4	3.2 cm	7.2	12.3	2.	5.1 cm	8.1 cm	1.	3.8 cm	5.3 cm	--	1.7	3.3	--	1.1	2.1

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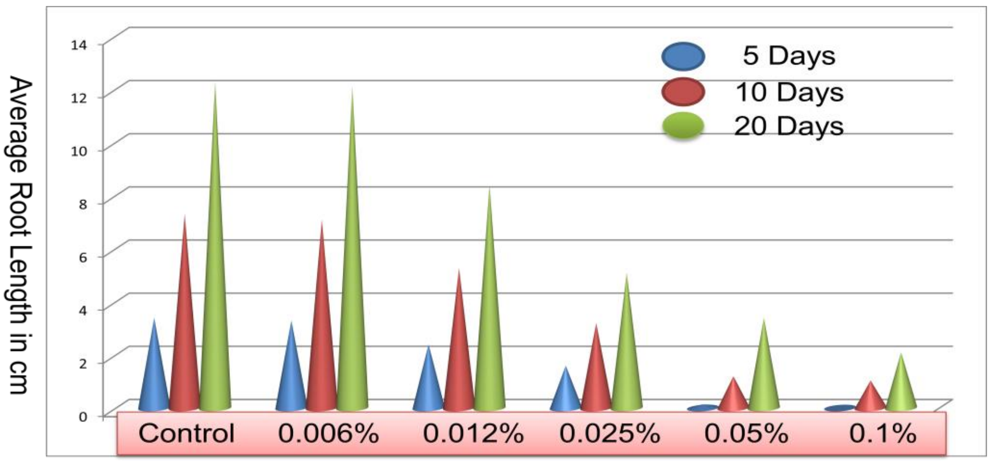
			cm		cm	cm	4 cm			8 cm				cm	cm		cm	cm
Surfexel	3.5 cm	6.9 cm	12.3 cm	3.5 cm	6.9 cm	12.2 cm	2.6 cm	5.1 cm	8.3 cm	1.7 cm	3.7 cm	5.2 cm	--	1.5 cm	3.2 cm	--	1.1 cm	2.2 cm
Sunflower	3.4 cm	7.4 cm	12.1 cm	3.3 cm	7.2 cm	12.2 cm	2.4 cm	5.4 cm	8.4 cm	1.9 cm	3.9 cm	5.6 cm	--	1.7 cm	3.2 cm	--	1.0 cm	2.4 cm
Wheel	3.5 cm	7.5 cm	12.5 cm	3.4 cm	7.2 cm	12.5 cm	2.7 cm	5.3 cm	8.5 cm	1.7 cm	3.7 cm	5.5 cm	--	1.6 cm	3.1 cm	--	1.1 cm	2.1 cm
Urvashi	3.6 cm	7.3 cm	12.3 cm	3.3 cm	7.3 cm	12.3 cm	2.3 cm	5.2 cm	8.7 cm	1.9 cm	3.8 cm	5.5 cm	--	1.8 cm	3.2 cm	--	1.2 cm	2.3 cm
XXX	3.2 cm	7.1 cm	11.9 cm	3.1 cm	7.0 cm	11.8 cm	2.1 cm	5.1 cm	8.1 cm	--	1.9 cm	4.1 cm	--	1.1 cm	2.7 cm	--	0.8 cm	1.7 cm
Average	**3.4 cm**	**7.3 cm**	**12.3 cm**	**3.3 cm**	**7,1 cm**	**12.1 cm**	**2.4 cm**	**5.27 cm**	**8.4 cm**	**1.6 cm**	**3.2 cm**	**5.1 cm**	**.14 cm**	**1.2 cm**	**3.4 cm**	**00 cm**	**1.04 cm**	**2.1 cm**

* Only Root length Mentioned here for Simplification Purpose; D* = Days

Fig-6: Root Length over time intervals



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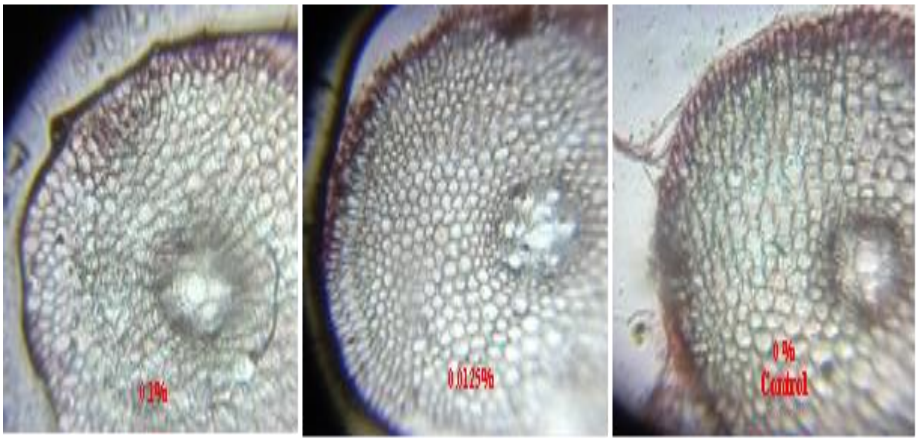
TS of Control roots possessed evenly arranged barrel shaped epidermal cells with numerous root hairs and its cortex contained loosely arranged parenchymal cells with prominent intracellular spaces. Whereas in the TS of roots generated in the detergents possessed irregularly arranged epidermal cells with no root hairs. Also the cortex contained compactly arranged heterogeneous cells. The endodermis of the roots generated in the detergents possessed thicker casparian bands than that of the control root endodermis. Enlarged pith region was observed in roots generated in detergents in comparison to the control roots as shown in fig-7.

Fig-7: foldscope images of onion roots TS generated in Control & Detergent solutions



Discussion

Detergents are inevitable chemical substances in day to day human life, but populace has least concern on their disposal, associated health hazards and negative impacts on environment especially in agriculture (Seyyed Alireza Mousavi and Farank Khodadoost; 2019). Very few studies described the impact of various types of detergents on agriculture and its productivity also with conflicted conclusions. Some studies described that detergents at low concentration promote crop growth and at high concentrations inhibit (Ehilen, O.E et.al; 2017, Syeda Uzma et.al; 2018). Other studies showed that detergents usually promote crop growth and productivity by increasing water and nutrients absorption of roots (Poongodi and Sasikala 2013). But in our study we used variety of regular detergents to study its influence on crop growth by taking organogenesis in onions as study model. For each detergent, we used a range of concentrations starting with minimum of 0.006%, surprisingly all tested detergents have negative impact on the organogenesis as compared with the control. The detergents showed negative impact on the root and shoot density, their length and also in their anatomy and morphology. In this study we also found that detergents significantly affect organogenesis in later days than early days shown in fig-5. With the study it can be presumed that detergents may have influence on the plant growth regulators and suppress the organogenesis. The study also highlighted the significance of considering the detergent effect while developing high yield crop varieties. The uniqueness of the study is the usage of low cost, origami based paper microscope i.e. foldscope for anatomical studies of onion roots grown in detergents, emphasized the educational and research potential of foldscope.

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Authors' Contribution

Conceptualization and designing of the research work (PM); Execution of field/lab experiments and data collection (HP); Analysis of data and interpretation, preparation of manuscript (KM, HR, SDC & MAK).

** PM = Pradeep Madhamanchi; HP = Hemanth Paidi; KM = Kishore Madhamanchi; HR = Haritha Ronanki; SDC = Shanthi Devi Chikile; MAK = M. Abdul Kareem*

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