Journal of Environmental Studies [JES]
An International Journal
https://jesj.journals.ekb.eg/
doi: 10.21608/JESJ.2022.123571.1004

Cytotoxicity of different concentrations of oxalic acid on onion cells

Ahmed R. Ali*, Ahmed M. M. A. Kasem, Abd El-Rehim A. El-Shanawany

Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assuit 71524, Egypt.

Citation: *Ahmed R., Ahmed M., Abdel-Rehim A., (2022). Cytotoxicity of different concentrations of oxalic acid on onion cells. Journal of Environmental Studies, Vol. 26(1): 1-8.*

Article Information

Received 23 Feb. 2022,
Revised 24 Feb 2022,
Accepted 27 Feb 2022.
Published online
30 June 2022

Abstract: The present study aimed to explore the effect of oxalic acid (OA) different concentrations (1, 2 and 4 g/L) and *Sclerotium cepivorum* crude extract on the cytology of *Allium cepa* germinating roots during four durations 6, 12, 18 and 24 hrs. The rate of cell division and mitotic chromosomal behavior in root tips of germinated bulbs beside the numbers and types of chromosomal abnormalities were recorded for the selected samples during the period of treatments after 6, 12, 18, 24 hrs by oxalic acid 1, 2, 4 g, and *S. cepivorum* crude extract, The results showed that the mitotic indices were reduced when the roots were treated by different concentrations of oxalic acid and *S. cepivorum* crude extract, and the reductions are proportional with both concentration and time. Also, several chromosomal abnormalities were recorded that includes vacuolated nuclei, rod-shape, binucleated cells, disrupted nuclei at interphase and lagged chromosomes, bridge, polyploidy, mega cell, star metaphase, mini-chromosomes, ring chromosomes, and deformed nuclei during mitosis. Results suggested that the oxalic acid affects pH value, Ca^{+} depletion and plasma membrane maceration, and it induces programmed cell death in onion.

Keywords: Mitotic activities, *Allium cepa*, *Sclerotium cepivorum*, Oxalic acid, Chromosomal abnormalities, Mitotic Index.

Introduction

Onion (*Allium cepa* L.) is an important horticultural crop in Egypt. According to the latest report issued by the Agricultural Export Council, Egypt's exports of fresh onions reached to 302,549 tons during the export season 2020/2021. The onion crop is affected by many fungal and bacterial diseases at different growth stages which result in considerable losses in yield. For several years, the ways of controlling diseases were dependent on excessive use of chemicals that cause various environmental and health related problems and deleterious effects on plant growth, development, and other metabolic activities (Sengupta *et al*., 1989; Pankratz *et al*., 2003). Also, some chemicals might undergo various changes and become more toxic, mutagenic and affect plants, animals and human (Grigorenko & Larchenko, 2000; Bolognesi 2003; Amaroli *et al*., 2013). Due to that, research now focus on understanding the mechanisms of disease and knowing all about the interactions that occur between the causative agent of the disease and the host (Pathakumari *et al*., 2020).

One of the main and most danger fungal disease which affects the onion crop and causes yield loss up to 100% is Allium White Rot disease worldwide and in Egypt (Yesuf, 2013), which caused by the soil-borne fungus *S. cepivorum* Berk. which produce small sclerotia serve as both propagules and inoculum due to the absence of a recognizable teleomorphic state (Couch & Kohn, 2000). These sclerotia can live and survive in the soil for more than 20 years and the germination can be occurred in response to the presence of onion sulfides to produce an infective mycelium of *S. cepivorum* (Coly-Smith *et al*., 1990). S. cepivorum can penetrate onion stem and grow inter and intracellular parenchyma causing cortical tissue disintegration and maceration of vascular tissue which cause a rapid watery rot of onion bulb scale (Abd-El-Razik *et al*., 1973). According to Bateman (1964), the maceration of host tissue by fungi depends on the fungal production of cell wall degrading enzymes, but in case of onion infection by *S. cepivorum*, oxalic acid is an essential factor beside the polygalacturonases to produce successful pathogenesis (Kritzman & Henis, 1977). The maceration of onion due to the infection by *S. cepivorum* is conducted by the synergistic action of endopolygalacturonase and oxalic acid (Stone & Armentrout, 1985).

* Corresponding author E-mail: ahmedram80@azhar.edu.eg

This study aims to determine the effect of different concentrations of oxalic acid on onion root cells during different exposure periods versus the effect of *S. cepivorum* Berk. exo-secretion extract during the same exposure periods.

Material and Methods

Allium Cepa Giza 6 bulbs were purchased from The National Center for Agricultural Research, Dokki, Giza, Egypt. *S. cepivorum* identified isolate was obtained from Plant Pathology Department, Faculty of Agriculture, Mansoura University, Egypt. Isolate was re-germinated on modified onion root potato dextrose (PD) liquid media by supporting the PD broth with 50% per weight of onion roots instead of potato to induce the production of exo-secretion and incubated at 16o C for 12 days in dark condition. Fungal extract was obtained by filtration to remove the mate of fungus and the filtrate was used as crude extract.

For cytological studies, selected bulbs were surface sterilized and grown in sterilized distilled water for 4 days. Then, the bulbs were transferred into 1, 2, and 4 g/L of oxalic acid solutions, and a crude extract solution of S. cepivorum solutions for 6, 12, 18 and 24 hrs. Cytological preparations were carried out according to Hiremath and Chinnappa (2015) by fixation of excised roots from each treatment in Carnoy's fixing solution of a mixture of glacial acetic acid and ethanol (1:3) for 2h. The fixed roots were hydrolyzed in 1N HCl for 8 min at 60 ^{O}C, then washed thoroughly with distilled water and moved to Feulgen staining solution for 10 min. at room temperature in dark condition until the tissue stains deep purple. the stained root tips were then cut and squashed separately on clean glass slides in a drop of 50 % glacial acetic acid solution and covered gently with clean covered slip and sealed with nail polisher. The slide visualization and cell count were done and photographed using Tucsen TCC-6.1ICE 6.1 Megapixel 12-bit Cooled Color CCD Microscope Camera.

Several cytological parameters were calculated from 10 different slides for each treatment (approximately 500 cells per slide) by the following equations:

Mitotic index (**MI**) = (number of divided cells)/ (number of total counted cells) ×100.

Phase index = (number of divided cells per phase)/ (number of total counted cells) ×100.

Relative Division Ratio = (MI treatment-MI control)/ (100-MI control) ×100.

Relative abnormality (**RDR**) = (number of abnormal cells) / (number of divided cells) ×100.

Absolute abnormality = (number of abnormal cells) / (number of total counted cells) ×100.

Chromosomal aberrations were recorded as vacuolated cell, rod-shaped, disrupted nucleus prophase, budded nucleus, binucleated, mega cell, star metaphase, lagging metaphase, lagging anaphase, bridge anaphase, polyploidy, lagging telophase and bridge telophase, and represented as percentage of dividing cells. All experiments were recited thrice, and the software SPSS was used for statistical analysis.

Results

The rate of cell division, mitotic chromosomal behavior in root tips of germinated bulbs and the numbers and types of chromosomal abnormalities were recorded for the selected samples during the period of treatments after 6, 12, 18, 24 hrs by oxalic acid 1, 2, and 4 g/L and *S. cepivorum* crude extract (Table 1, Fig. 1, 2).

The results after 6 h of treatments revealed that mitotic index of control roots has the highest value (20.3%), and the MI were reduced to 13.5, 10, 0.4, and 8% when the roots treated by 1, 2, 4 g OA and *S. cepivorum* crude extract, respectively. The RDR were decreased to 8.4,12.9, 14 and 24.9%, respectively. The interphase normal cells decreased from 86.4% in control to 81.1%, 78.5%, and 73.9%, and 12.9%, respectively.

Chromosomal Aberrations recorded in the interphase in this period were: vacuolated nuclei (9.2, 18, 18.6, 0.4 and 20.0%), rod-shape cell (4.4, 2.0, 6.3, 0.0 and 0.3%), binucleated cells (0.0, 0.5, 1,2, 19.6 and 4.4%) in of interphase cells in control, 1, 2, 4 g of oxalic acid and *S. cepivorum* crude extract, respectively. The disrupted nuclei were recorded only in the roots treated by *S. cepivorum* crude extract (60.7% of interphase cells). The chromosomal aberrations in the interphase cells by the end of the first period were 13.6, 18.9, 21.5, 26.1 and 87.1%, respectively. The prophase indices were 13.5, 8.3, 6, 0.2 and 0.3%, respectively, the metaphase indices were 2.0, 1.7, 1.7, 0.2 and 0.1%, respectively, the anaphase indices were 1.3, 0.9, 0.9, 0.0 and 0.4%, respectively, the telophase indices scored 3.4, 2.6, 1.4, 0.0 and 0.0%, respectively.

The mitotic abnormalities recorded lagging metaphase (2.8, 9.2, 14.6, 20.0 and 14.6%, respectively), lagging anaphase (0.0, 2.3, 1.8, 0.0 and 26.8%, respectively), bridge anaphase (3.8, 2.1, 6.6, 0.0 and 19.5%), polyploidy (0.0, 0.5, 2.0, 0.0, 0.0%), lagging telophase (0.0, 17.4, 13, 0.0 and 0.0%, respectively), mega cell (up to 7.3% at 2 g OA), star metaphase (30% at 4 g OA), and bridge telophase (0.5% at control only), and the percentages of mitotic aberrations were 9.3, 33.7, 43.3, 50.0 and 61.0%, respectively.

After 12 hrs of treatments, mitotic index of control roots had the highest value (21.6%), and the MI were reduced to 18.4%, 5.3%, and 4.8% when the roots treated by 1, 2 and 4 g/L of oxalic acid, respectively. The lowest

value of MI was 0.2% which recorded in the roots that treated by *S. cepivorum* crude extract. The RDR were decreased to 4, 20.7, 21.4 and 27.3, respectively. The interphase normal cells decreased from 87.3% in control to 77.4%, 75.4%, and 49.6%, and 38.8%, respectively. Chromosomal Aberrations recorded in the interphase in this period were: vacuolated nuclei (8.3, 19.8, 20.35, 1.8 and 8.2%), rod-shape cell (3, 2.1, 1.6, 0.0 and 3%), binucleated cells (0.0, 1.7, 28.0, 19.2 and 7.3%), and disturbed nuclei were recorded only in the roots treated by *S. cepivorum* crude extract (41.9% of interphase cells).

The chromosomal aberrations in the interphase cells by the end of the second period were 12.7, 24.6, 50.4, 22.6 and 50.4% at control, 1, 2, 4 g OA and *S. cepivorum* crude extract , respectively. The prophase indices were 14.2, 13.7, 3.0, 1.9 and 0.0%, respectively. The metaphase indices were 2.2, 1.9, 0.5, 1.6 and 0.2%, respectively. The anaphase indices were 1.4, 0.8, 0.9, 0.5 and 0.0%, respectively. The telophase indices scored 3.7, 2.0, 1.0, 0.7 and 0.0%, respectively.

The mitotic abnormalities noticed in all treated include lagging metaphase (3.4, 10.3, 9.0, 33.9 and 100%, respectively), lagging anaphase (0.0, 0.6, 0.0, 0.0 and 0.0%, respectively), bridge anaphase (2.6, 2.1, 12.6, 10.4 and 0.0%), polyploidy (0.9, 1.9, 4.7, 0.0, 0.0%), lagging telophase (0.0, 9.9, 18.0, 15.1 and 0.0% , , respectively), mega cell (up to 1.6% at controlled roots), star metaphase (0.0% at control and treated roots), and bridge telophase (0.5% at control only). The percentages of Mitotic aberrations achieved were 9.1, 24.7, 44.2, 59.4 and 100%, respectively.

The results after 18 hrs of treatments revealed that; Mitotic index of control roots has the highest value (20.7%), and the MI were reduced to 17.2%, 6.3%, and 5.7% when the roots treated by 1, 2, 4 g of oxalic acid, respectively, the lowest value of MI was 0.4% which recorded in the roots that treated by *S. cepivorum* crude extract. The RDR were decreased to -4.4%, -18.1%, -18.9% and -26.1%, respectively. The interphase normal cells decreased from 86.4% in control to 49.8%, 38.8%, 47.5%, and 41.1%, respectively. Chromosomal Aberrations recorded in the interphase in this period were: vacuolated nuclei (9.2, 22.2, 41.4, 32.9 and 10%), rod-shape cell (4.4, 8.1, 2.8, 4.6 and 5.1%), binucleated cells (0.0, 7.9, 39.1, 15 and 5.3%) in of interphase cells in control, 1, 2, 4 g of oxalic acid and *S. cepivorum* crude extract, respectively. The disrupted nuclei were recorded only in the roots treated by *S. cepivorum* crude extract (38.5% of interphase cells); The budded nuclei were recorded only in the roots treated by 1 g O acid (12% of interphase cells).

The chromosomal aberrations in the interphase cells by the end of the third period were 13.6, 50.2, 83.2, 52.5 and 58.9% at control, 1, 2, 4 g OA and *S. cepivorum* crude extract, respectively.

The prophase indices were 13.6, 13.0, 2.4, 3.1 and 0.0%, respectively. The metaphase indices were 2.0, 1.7, 1.5, 1.1 and 0.0%, respectively. The anaphase indices were 1.4, 0.4, 0.8, 0.7 and 0.0%, respectively. The telophase indices scored 3.7, 2.0, 1.6, 0.8 and 0.0%, respectively. The mitotic abnormalities noticed in all treated include lagging metaphase (2.7, 10, 24.3, 19.9 and 0.0%, respectively), lagging anaphase (0.0, 0.6, 0.0, 0.0 and 0.0%, respectively), bridge anaphase (3.6, 0.8, 12, 12.6 and 0.0%), polyploidy (0.6, 1.2, 0.0, 0.0, 0.0%), lagging telophase (0.0, 10.7, 25.5, 14 and 0.0%, respectively), mega cell (1.6, 0.0, 8.9, 0.7, 0.0%), and bridge telophase (0.8% at control only). The percentages of Mitotic aberrations achieved were 9.4, 23.3, 70.8, 47.2 and 0.0%, respectively.

The results after 24 hrs of treatments revealed that; Mitotic index of control roots has the highest value (20.1%), and the MI were reduced to 9.4%, 6.3%, and 6.6% when the roots treated by 1, 2 and 4 g of oxalic acid, respectively, the lowest value of MI was 0.0% which recorded in the roots that treated by *S. cepivorum* crude extract. The RDR were decreased to -13.3%, -17.2%, -16.8% and -25.1%, respectively. The interphase normal cells decreased from 86.4% in control to 78.9%, 28.5%, 68.7%, and 27.1%, respectively. Chromosomal Aberrations recorded in the interphase in this period were: vacuolated nuclei (9.2, 13.1, 18.7, 9.7 and 0.0%), rod-shape cell (4.4, 3.3, 4.1, 2.9 and 0.0%), binucleated cells (0.0, 4.6, 18.7, 18.6 and 2.1%) in of interphase cells in control, 1, 2, 4 g of oxalic acid and *S. cepivorum* crude extract, respectively. The disrupted nuclei were recorded only in the roots treated by *S. cepivorum* crude extract (70% of interphase cells). The chromosomal aberrations in the interphase cells by the end of the fourth period were 13.6, 21.1, 71.5, 31.3 and 72.9% at control, 1, 2, 4 g OA and *S. cepivorum* crude extract, respectively.

The prophase indices were 13.5, 7, 2.9, 3.4 and 0.0%, respectively. The metaphase indices were 2.0, 1.3, 1.9, 1.4 and 0.0%, respectively. The anaphase indices were 1.2, 0.2, 0.5, 0.9 and 0.0%, respectively. The telophase indices scored 3.4, 0.9, 1.0, 1.0 and 0.0%, respectively. The mitotic abnormalities noticed in all treated include lagging metaphase (2.6, 13.5, 30.8, 20.5 and 0.0% , respectively), lagging anaphase (0.0, 2.4, 0.0, 5.9 and 0.0% , respectively), bridge anaphase (3.4, 0.0, 7.7, 7.9 and 0.0%), polyploidy (up to 0.4% in controlled roots), lagging telophase (0.0, 10.1, 15.4, 14.7 and 0.0% , respectively), mega cell (1.4, 24.4, 3.1, 2.6 and 0.0% , respectively), and bridge telophase (0.4% at control only). The percentages of mitotic aberrations recorded were 8.1, 5o.3, 56.9, 51.6 and 0.0%, respectively.

Table 1: Chromosomal aberrations in root tips of *Allium cepa* induced by 1, 2, and 4 g/L of oxalic acid, and *Sclerotium cepivorum* crude extract at 6, 12, 18, 24 h exposure periods

	treatments	Total Counted Cell	To no. Interphase cells	Interphase						To. Int. ph. Abnormality	Mitotic Division						Mitotic Aberrations						To. Mitotic Abnormality	Total Abnormality	Relative Abnormality	Absolute Abnormality	Mitotic Index	RDR
				Normal Cell	Vacuolated Cell	Rod shape Cell	Disrupted Nucleus	Budded Nucleus	Binucleated		Prophase	Metaphase	Anaphase	Telophase	Mega cell	Star Metaphase	Lagging Metaphase	Lagging Anaphase	Bridge Anaphase	Ploidy	Lagging Telophase	Bridge Telophase						
6 h	Control	5283	4213	3639	389	185	0	0	0	574	697	75	22	177	18	0	30	0	41	5	0	5	99	673	12.7	1.9	20.3	
	%			86.4	9.2	4.4	0.0	0.0	0.0	13.6	13.5	2.0	1.3	3.4	1.7	0.0	2.8	0.0	3.8	0.5	0.0	0.5	9.3					
	Ox 1 gm	5200	4497	3649	810	15	0	0	23	848	433	19	0	14	0	5	65	16	15	14	122	0	237	1085	20.9	4.6	13.5	-8.4
	%			81.1	18.0	0.3	0.0	0.0	0.5	18.9	8.3	1.7	0.9	2.6	0.0	0.7	9.2	2.3	2.1	2.0	17.4	0.0	33.7					
	Ox 2 gm	5480	4933	3647	916	310	0	0	60	1286	290	13	3	4	40	0	80	10	36	0	71	0	237	1523	27.8	4.3	10.0	-12.9
	%			73.9	18.6	6.3	0.0	0.0	1.2	26.1	6.0	1.7	0.9	1.4	7.3	0.0	14.6	1.8	6.6	0.0	13.0	0.0	43.3					
	Ox 4 gm	5165	5145	4037	100	0	0	0	1008	1108	10	0	0	0	0	6	4	0	0	0	0	0	10	1118	21.6	0.2	0.4	-24.9
	%			78.5	1.9	0.0	0.0	0.0	19.6	21.5	0.2	0.2	0.0	0.0	0.0	30.0	20.0	0.0	0.0	0.0	0.0	0.0	50.0					
	Sc. Cep.	5062	5021	650	1003	98	3050	0	220	4371	16	0	0	0	0	0	6	11	8	0	0	0	25	4396	86.8	0.5	0.8	-24.4
	%			12.9	20.0	2.0	60.7	0.0	4.4	87.1	0.3	0.1	0.4	0.0	0.0	0.0	14.6	26.8	19.5	0.0	0.0	0.0	61.0					
12 h	Control	5406	4240	3700	350	190	0	0	0	540	750	80	35	195	19	0	40	0	30	10	0	7	106	646	11.9	2.0	21.6	
	%			87.3	8.3	4.5	0.0	0.0	0.0	12.7	14.2	2.2	1.4	3.7	1.6	0.0	3.4	0.0	2.6	0.9	0.0	0.6	9.1					
	Ox 1 gm	5260	4290	3235	850	130	0	0	75	1055	720	0	0	10	0	0	100	6	20	18	96	0	240	1295	24.6	4.6	18.4	-4.0
	%			75.4	19.8	3.0	0.0	0.0	1.7	24.6	13.7	1.9	0.8	2.0	0.0	0.0	10.3	0.6	2.1	1.9	9.9	0.0	24.7					
	Ox 2 gm	5235	4957	2460	1005	102	0	0	1390	2497	155	0	0	0	0	0	25	0	35	13	50	0	123	2620	50.0	2.3	5.3	-20.7
	%			49.6	20.3	2.1	0.0	0.0	28.0	50.4	3.0	0.5	0.9	1.0	0.0	0.0	9.0	0.0	12.6	4.7	18.0	0.0	44.2					
	Ox 4 gm	5244	4993	3864	90	80	0	0	959	1129	102	0	0	0	0	0	85	0	26	0	38	0	149	1278	24.4	2.8	4.8	-21.4
	%			77.4	1.8	1.6	0.0	0.0	19.2	22.6	1.9	1.6	0.5	0.7	0.0	0.0	33.9	0.0	10.4	0.0	15.1	0.0	59.4					
	Sc. cep.	5095	5086	1974	416	190	2133	0	373	3112	0	0	0	0	0	0	9	0	0	0	0	0	9	3121	61.3	0.2	0.2	-27.3
	%			38.8	8.2	3.7	41.9	0.0	7.3	61.2	0.0	0.2	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0	100.0					
18 h	Control	5364	4255	3675	393	187	0	0	0	580	710	76	30	189	18	0	30	0	40	7	0	9	104	684	12.8	1.9	20.7	
	%			86.4	9.2	4.4	0.0	0.0	0.0	13.6	13.6	2.0	1.4	3.7	1.6	0.0	2.7	0.0	3.6	0.6	0.0	0.8	9.4					
	Ox 1 gm	5221	4323	2153	960	350	0	520	340	2170	678	0	0	11	0	0	90	5	7	11	96	0	209	2379	45.6	4.0	17.2	-4.4
	%			49.8	22.2	8.1	0.0	12	7.9	50.2	13.0	1.7	0.4	2.0	0.0	0.0	10.0	0.6	0.8	1.2	10.7	0.0	23.3					
	Ox 2 gm	5125	4800	807	1985	133	0	0	1875	3993	95	0	0	0	29	0	79	0	39	0	83	0	230	4223	82.4	4.5	6.3	-18.1
	%			16.8	41.4	2.8	0.0	0.0	39.1	83.2	2.4	1.5	0.8	1.6	8.9	0.0	24.3	0.0	12.0	0.0	25.5	0.0	70.8					
	Ox 4 gm	5270	4969	2358	1637	230	0	0	744	2611	159	0	0	0	2	0	60	0	38	0	42	0	142	2753	52.2	2.7	5.7	-18.9
	%			47.5	32.9	4.6	0.0	0.0	15.0	52.5	3.1	1.1	0.7	0.8	0.7	0.0	19.9	0.0	12.6	0.0	14.0	0.0	47.2					
	Sc. Cep	5140	5140	2110	516	260	1981	0	273	3030	0	0	0	0	0	0	0	0	0	0	0	0	0	3030	58.9	0.0	0.0	-26.1
	%			41.1	10.0	5.1	38.5	0.0	5.3	58.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					
24 h	Control	5164	4128	3566	381	181	0	0	0	562	683	74	22	173	14	0	27	0	35	4	0	4	84	646	12.5	1.6	20.1	
	%			86.4	9.2	4.4	0.0	0.0	0.0	13.6	13.5	2.0	1.2	3.4	1,4	0.0	2.6	0.0	3.4	0.4	0.0	0.4	8.1					
	Ox 1 gm	5283	4786	3775	629	160	0	0	222	1011	247	0	0	0	121	0	67	12	0	0	50	0	250	1261	23.9	4.7	9.4	-13.3
	%			87.9	13.1	3.3	0.0	0.0	4.6	21.1	7.0	1.3	0.2	0.9	24.3	0.0	13.5	2.4	0.0	0.0	10.1	0.0	50.3					
	Ox 2 gm	5150	4825	1375	900	200	0	0	2350	3450	140	0	0	0	10	0	100	0	25	0	50	0	185	3635	70.6	3.6	6.3	-17.2
	%			28.5	18.7	4.1	0.0	0.0	48.7	71.5	2.9	1.9	0.5	1.0	3.1	0.0	30.8	0.0	7.7	0.0	15.4	0.0	56.9					
	Ox 4 gm	5170	4829	3319	470	140	0	0	900	1510	165	0	0	0	9	0	70	20	27	0	50	0	176	1686	32.6	3.4	6.6	-16.8
	%			68.7	9.7	2.9	0.0	0.0	18.6	31.3	3.4	1.4	0.9	1.0	2.6	0.0	20.5	5.9	7.9	0.0	14.7	0.0	51.6					
	Sc. cep.	5221	5221	1417	0	0	3654	0	150	3804	0	0	0	0	0	0	0	0	0	0	0	0	0	3804	72.9	0.0	0.0	-25.1
	%			27.1	0.0	0.0	70.0	0.0	2.9	72.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0					

Discussion

Oxalic acid is a virulence factor of several phytopathogenic fungi but the detailed mechanisms by which oxalic acid affects host cells and tissues are not fully understood. OA is widely existing in many biological systems and several studies have shown that the exogenous application of OA mimicked fungal disease prevalence (Qi *et al*., 2017). The secretion of OA was essential to affect pathogenicity in plants by Sclerotium spp. fungal pathogen infection (Williams *et al*., 2011) and it has been recorded as an effective elicitor and improving plant resistance (Dong *et al*., 2008; Monazzah *et al*., 2018; Sun *et al*., 2019).

Previous results demonstrated the accumulation of OA can acidify the infected plant tissues to activate many fungal enzymes and protein kinase of host plant cells at low pH and degrade the plant cell wall via acidity or chelation of the cell wall Ca^{2+} (Grabski *et al*., 1994; Boller, 1995; Kumar *et al*., 2021) and the application of exogenous OA modulated the distribution of Ca^{2+} (Sadak & Orabi, 2015; Li *et al*., 2016). This was evident in in the type of chromosomal abnormalities in our results, OA depletion of Ca^{2+} leads to spindle fibers malfunction abnormalities such as star metaphase, lagged chromosomes, chromosomal bridge, polyploidy, sticky, and disturbed chromosomes. These abnormalities were increased by increasing the concentration of OA and the time of treatments. The rates of cell division were decrease slightly at low concentrations within the least time duration, but with increasing either the applied concentration or time interval, the reduction was greatly increased. Also, it has been observed that the dynamic changes in Ca^{2+} spatial and temporary distribution might correlate closely with its distinct roles played during programmed cell death of plants or other plant physiology processes (Borrelli *et al*., 2016; Gębura & Winiarczyk 2016).

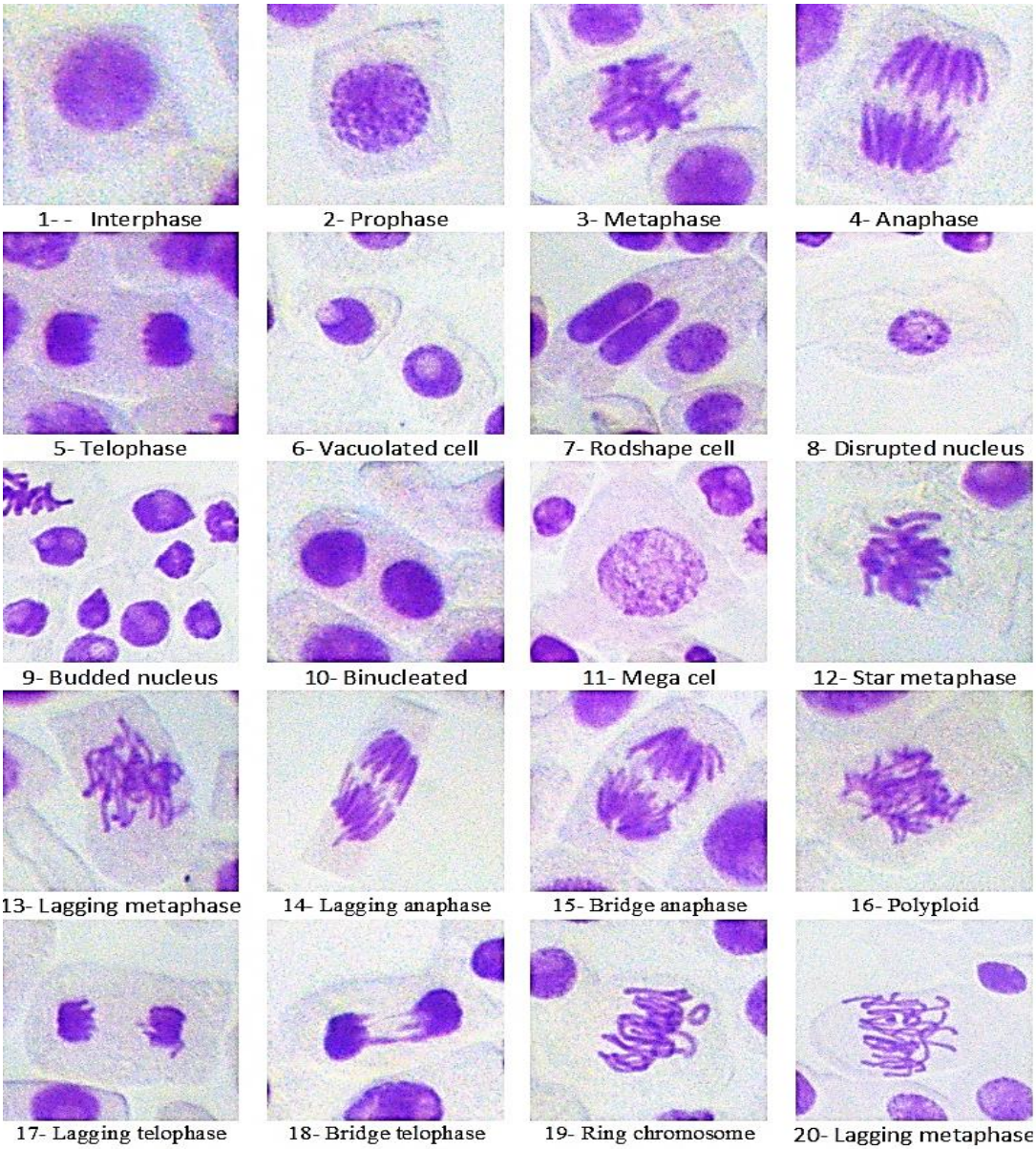

Fig. 1: Different chromosomal aberrations in root tips of *Allium cepa* (1-20) induced by 1, 2, and 4 g/L of oxalic acid, and *Sclerotium cepivorum* crude extract.

The OA appears to function during plant-microbe interaction by triggering the pathways responsible for programmed cell death in plants (Ravi *et al*., 2017; Fagundes-Nacarath *et al*., 2018). OA induces cytoplasmic acidification which triggers the synthesis of phytoalexins and other secondary metabolites (Westphal *et al*., 2019). The cytoplasmic acidification caused DNA breakdown which is evident in the type of chromosomal break, ring chromosome, lagged chromosomes and micronuclei in our results.

Intracellular acidification, combined with K^+ and Ca^{2+} flux, was regarded as an early marker of an elicitation process leading to PCD. Several studies suggested that changes in cytoplasmic pH resulting from ion fluxes and H^+-ATPase play a role (Afzal *et al*., 2020). This may explain the sever reduction in mitotic index at all time durations treatments of higher OA concentrations and *Sclerotium* extract, and the appearance of deformed nuclei and dead cells. These results might suggest that the addition of OA played inhibition role on the inward K^+ current and the accumulated H^+ in the cytoplasm thus altered the activity of the K^+/H^+ exchanger. The Alteration in H^+ in the early response of plant cells to environmental stimuli, such as turgor, gravity, pathogen attack and chemicals exposure, have been well explored (Gonugunta *et al*., 2009).

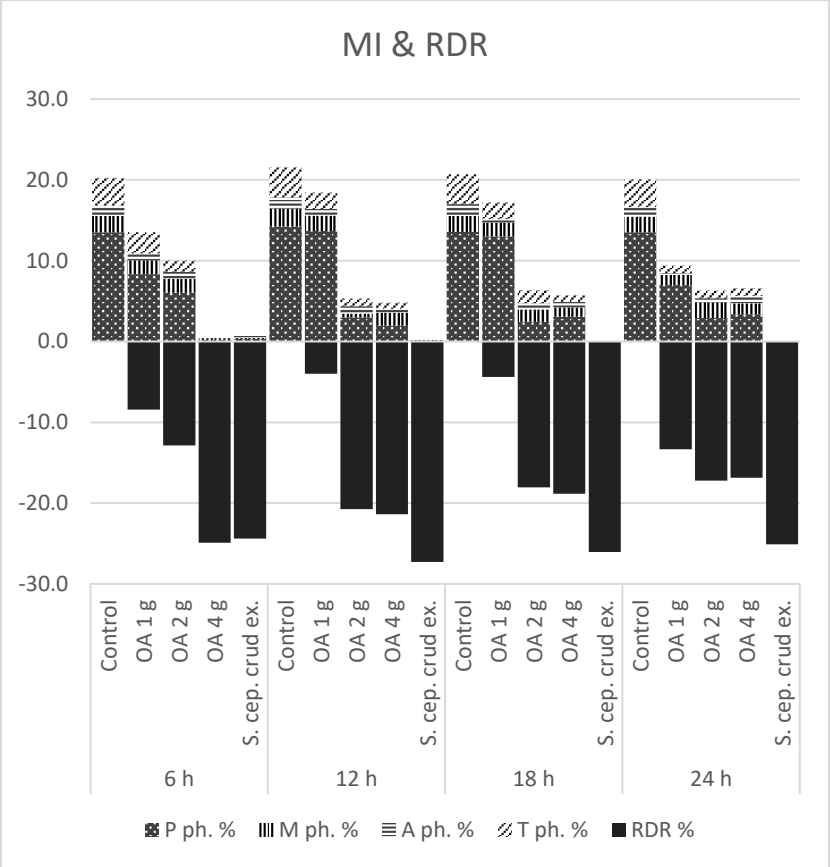

Fig. 2: Index rates of mitotic phases in root tip cells of *Allium cepa* after 6, 12, 18, and 24 hrs exposure to 1, 2, and 4 g/L of oxalic acid, and *Sclerotium cepivorum* crude extract.

Many physiological events of plant cells, such as nutrient transport across the plasma membrane, cell elongation, and organ development, are highly dependent on the ability of individual cells to control pH both in cytosol and apoplast (Staal *et al*., 2011). Generally, the modulation of intracellular pH or extracellular pH could lead to depolarization or hyperpolarization in the plasma membrane (Wang *et al*., 2018). They were subsequently followed by triggering or inhibiting a series of physiological events at the plasma membrane, such as control of ion channels activities, signaling and nutrient uptake, and cell growth (Zhang *et al*., 2005). In our results, the transduction of such signals leading to the death of onion cells in response to OA treatment which was evident by the decrease of the normal cells and the increase cell mortality at higher OA concentrations. This death displayed characteristic hallmarks of PCD, such as cell shrinkage, deformed nuclei, cleavage of nuclear DNA, and activation of anion channel-dependent, and gene expression (Errakhi *et al*., 2008). In this study, we confirmed that high concentrations of OA induced PCD in onion cells. The effect of oxalic acid mimics S. cepivorum crude extract on the cytology of Allium cepa germinating roots and the results showed that Mitotic indices were reduced, and the reductions were proportional with both concentration and time. Chromosomal abnormalities related to spindle fiber and protein destruction, Ca ion depletion, DNA break, pH reduction was recorded such as vacuolated nuclei, rod-shape cell, binucleated cells, disrupted nuclei lagged chromosomes, bridge, chromosome polyploidy, mega cell, star metaphase, minichromosomes, ring chromosomes, and deformed nuclei during mitosis.

References

Abd-El-Razik, A. A., Shatla, M. N., Rushdi, M. (1973). Studies on the infection of onion plants by *Sclerotium cepivorum* Berk. Phytopathology, 76: 108–116.

Afzal, M. R., Zhang, M., Jin, H., Wang, G., Zhang, M., Ding, M., Raza, S., Hu, J., Zeng, H., Gao, X. (2020). Post-translational regulation of plasma membrane H^+-ATPase is involved in the release of biological nitrification inhibitors from sorghum roots. Plant Soil, 450: 357–372.

Amaroli, A., Aluigi, M., Falugi, C., Chessa, M. G. (2013). Effects of neurotoxic thionophosphate pesticide chlorpyrifos on differentiating alternative models. Chemosphere, 90: 2115–2122.

Armentrout, V. N., Stone, H. E. (1985). America Production of Oxalic Acid by *Sclerotium cepivorum* during Infection of Onion. Mycologia, 77(4): 526–530.

Bateman, D. F. (1964). An induced mechanism of tissue resistance to polygalacturonase in *Rhizoctonia* infected hypocotyls of bean. Phytopathology, 53: 197–204.

Boller, T. (1995). Chemoperception of microbial signals in plant cells. Annual Review Plant Biology, 46: 189–214.

Bolognesi, C. (2003). Genotoxicity of pesticides: A review of human biomonitoring studies. Mutant Research, 543(3): 251–272.

Borrelli, N., Benvenuto, M. L., Osterrieth, M. (2016). Calcium oxalate crystal production and density at different phenological stages of soybean plants (*Glycine max* L.) from the southeast of the Pampean Plain, Argentina. Plant Biology, 1016–1024.

Coley-Smith, J. R., Mitchell, C. M., Sansford, C. E. (1990). Long-term survival of sclerotia of *Sclerotium cepivorum* and *Stromatinia gladioli*. Plant Pathology, 39: 58–69.

Couch, B. C., Kohn, L. M. (2000). Clonal spread of *Sclerotium cepivorum* in onion production with evidence of past recombination events. Phytopathology, 90: 514–521.

Dong, X., Ji, R., Guo, X., Foster, S. J., Chen, H., Dong, C., Liu, Y., Hu, Q., Liu, S. (2008). Expressing a gene encoding wheat oxalate oxidase enhances resistance to *Sclerotinia sclerotiorum* in oilseed rape (*Brassica napus*). Planta, 228: 331–340.

Errakhi, R., Meimoun, P., Lehner, A., Vidal, G., Briand, J., Corbineau, F., Rona, J. P. Bouteau, F. (2008). Anion channel activity is necessary to induce ethylene synthesis and programmed cell death in response to oxalic acid. Journal of Experimental Botany, 59: 3121–3129.

Fagundes-Nacarath, I., Debona, D., Rodrigues, F. (2018). Oxalic acid-mediated biochemical and physiological changes in the common bean *Sclerotinia sclerotiorum* interaction. Plant Physiology and Biochemistry, 129: 109–121.

Gębura, J., Winiarczyk, K. (2016). A study on calcium oxalate crystals in *Tinantia anomala* (Commelinaceae) with special reference to ultrastructural changes during another development. Journal of Plant Research, 129: 685–695.

Gonugunta, V. K., Srivastava, N., Raghavendra, A. S. (2009). Cytosolic alkalinization is a common and early messenger preceding the production of ROS and NO during stomatal closure by variable signals, including abscisic acid, methyl jasmonate and chitosan. Plant Signal Behaviour, 4: 561–564.

Grabski, S., Xie, X. G., Holland, J. F., Schindler, M. (1994). Lipids trigger changes in the elasticity of the cytoskeleton in plant cells: A cell optical displacement assay for live cell measurements. Journal of Cell Biololgy, 126: 713–726.

Grigorenko, N. V., Larchenko, E. A. (2000). Mutagenic effect of the herbicide Titus in maize. Tsitol Genetics, 34(5): 50–54.

Hiremath S., Chinnappa C. (2015). Plant Chromosome Preparations and Staining for Light Microscopic Studies. In: Yeung E., Stasolla C., Sumner M., Huang B. (eds) Plant Microtechniques and Protocols. Springer, New York, USA, pp263–286.

Kritzman, G., Chet, I., Henis, Y. (1977). The role of oxalic acid in the pathogenic behavior of *Sclerotium rolfsii* Sacc. Experimental Mycology, 1: 280-285.

Kumar, R., Kumar, R., Prakash, O., Kumar, R., Pant, A. (2021). Chemical Signal Dissemination Through Info. chemicals. In: Microbial Metatranscriptomics Belowground. Springer, New York, NY, USA, pp. 91–108.

Li, P., Yin, F., Song, L., Zheng, X. (2016). Alleviation of chilling injury in tomato fruit by exogenous application of oxalic acid. Food Chemistry, 202: 125–132.

Monazzah, M., Rabiei, Z., Enferadi, S. T. (2018). The effect of oxalic acid, the pathogenicity factor of *Sclerotinia Sclerotiorum* on the two susceptible and moderately resistant lines of sunflower. Iranian Journal of Biotechnology, 16: e1832.

Pankratz, F. B., Doebel, C., Farenhorst, A., Goldsborough, L. G. (2003). Interactions between algae (*Selenastrum capricornutum*) and pesticides: implications for managing constructed wetlands for pesticides removal.

Journal of Environmental Science Health, B 38:147.

Pathakumari, B., Liang, G., Liu, W. (2020). Review Immune defense to invasive fungal infections: A comprehensive review, Biomedicine & Pharmacotherapy, 130: 110550.

Qi, Z.,Yu, J., Shen, L., Yu, Z., Yu, M., Du, Y., Zhang, R., Song, T., Yin, X., Zhou, Y. (2017). Enhanced resistance to rice blast and sheath blight in rice (*Oryza sativa* L.) by expressing the oxalate decarboxylase protein Bacisubin from *Bacillus subtilis.* Plant Science, 265: 51-60

Ravi, K., Pareek, S., Kaushik, R., Ameta, K. (2017). Effect of Exogenous Application of Oxalic Acid on Physiological and Biochemical Characteristics in 'Gola'Ber (*Ziziphus mauritiana* Lamk.) Fruit during Storage. Research Journal of Agricultural Science, 8: 1249–1252.

Sadak, M. S., Orabi, S. A. (2015). Improving thermo tolerance of wheat plant by foliar application of citric acid or oxalic acid. International Journal of ChemTech Research, 8: 333–345.

Sengupta, P. K., Chakrabarti, A., Banerjee, S. L. (1989). Malathion-induced effects on growth and nitrogen metabolizing enzymes in germinating wheat. Current Science, 58: 554–556.

Staal, M., De Cnodder, T., Simon, D., Vandenbussche, F., Van Der Straeten, D., Verbelen, J.-P., Elzenga, T., Vissenberg, K. (2011). Apoplastic alkalinization is instrumental for the inhibition of cell elongation in the Arabidopsis root by the ethylene precursor 1-aminocyclopropane-1-carboxylic acid. Plant Physiology, 155: 2049-2055.

Sun, G., Feng, C., Zhang, A., Zhang, Y., Chang, D., Wang, Y., Ma, Q. (2019). The dual role of oxalic acid on the resistance of tomato against *Botrytis cinerea*. World Journal of Microbiology and Biotechnology, 35(2): 36.

Wang, Y., Blatt, M. R., Chen, Z. (2018). H Ion transport at the plant plasma membrane. eLS 1–16.

Westphal, L., Strehmel, N., Eschen-Lippold, L., Bauer, N., Westermann, B., Rosahl, S., Scheel, D., Lee, J. (2019). pH effects on plant calcium fluxes: Lessons from acidification-mediated calcium elevation induced by the γ-glutamyl-leucine dipeptide identified from Phytophthora infestans. Science Reports, 9: 1–14.

Williams, B., Kabbage, M., Kim, H. J., Britt, R., Dickman, M. B. (2011). Tipping the balance: *Sclerotinia sclerotiorum* secreted oxalic acid suppresses host defenses by manipulating the host redox environment. PLoS Pathogens, 7: e1002107.

Yesuf, M. (2013). Searching and evaluating of cost-effective management options of garlic white rot (*Sclerotium cepivorum* Berk) in Tigray, Northern Ethiopia. Journal of Plant Pathology & Microbiology, 4,7.

Zhang, Z., Ramirez, J., Reboutier, D., Brault, M., Trouverie, J., Pennarun, A. M., Amiar, Z., Biligui, B., Galagovsky, L., Rona, J. P. (2005). Brassinosteroids regulate plasma membrane anion channels in addition to proton pumps during expansion of Arabidopsis thaliana cells. Plant Cell Physiology, 46: 1494–1504.