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Comparative Study of the Cytotoxic Effects of Raw *Aloe vera* Gel Extract on the Root Tip Cells of *Allium cepa* and *Allium sativum*

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ABSTRACT: The *Aloe vera* plant is a succulent plant and its leaf is widely used in the field of cosmetic, Ayurvedic, Homeopathic Allopathic, as well as in food industry. The present study has been carried out to know about the cytotoxic effect of raw *A. vera* gel extract on the root tip cells of both *Allium cepa* (Onion) and *A. sativum* (Garlic). Three different concentrations of raw *A. vera* gel extract (i.e., 20%, 40% and 60%) were prepared for the experiment. The prepared microscopic slides were observed under a microscope. By summarizing the results using Analysis of Variance (ANOVA) it has been observed that the effect of 20%, 40% and 60% *A. vera* extract for the exposure of 24 hours on onion is higher than the garlic. Thus, the results of this study indicates that the raw *A. vera* gel extract exhibits some cytotoxic effect on both the root tip cells of *A. cepa* and *A. sativum*, but the effect of the raw gel extract is higher on onion root tip cells. *Aloe vera* has many beneficial effects and therefore, it was the most challenging part to establish its cytotoxic effect in terms of mitotic inhibition. Thus, we can conclude that the raw *A. vera* gel should not be consumed as it caused mitotic inhibition in both onion and garlic root tip cells.

Keywords: *Aloe vera*, Cytotoxic, *Allium cepa*, *Allium sativum*, Mitotic inhibition, Mitotic Index.

INTRODUCTION

Aloe vera is a popular medicinal herb, which is mainly a succulent plant and it grows in arid and subtropical climates. The *A. vera* plant belongs to the family-Asphodelaceae. It grows up-to a height of 60-100 cm (24-39 inches) and it generally matures in about 4-6 years and it can survive for a period of nearly 50 years under favorable climatic conditions (Lanka, 2018). It is observed that the edge of the leaf is serrated and has little white teeth. Like other *Aloe* species, *A. vera* shapes arbuscular mycorrhiza, a beneficial interaction that permits the plant to get mineral supplements from the soil in a better way. *A. vera* plant is popular all over the world because of its various medicinal properties. It is widely used in the field of Ayurvedic, Homeopathic and Allopathic stream of medical science (Lanka, 2018). *A. vera* is a natural product which is currently used in large scale in the field of cosmetology. As we all know that various creams, lotions, face washes, hair oils etc. which we regularly use in our daily life contains *A. vera* as the essential and main ingredient. The name *Aloe vera* derives from the Arabic word "*Alloeh*", which means "shinning bitter substance", while "*vera*" in Latin means "true". 2000 years ago, the Greek scientists regarded *A. vera* as the universal panacea, which means it can cure any disease. The Egyptians called *Aloe* "the plant of immortality". Today, the *A. vera* plant has also been widely used for various purposes in the field of dermatology (Surjushe *et al.,* 2008).

The *Aloe vera* plants have thick fleshy leaves which help the plant to store water in the form of gel. The leave of the plant is also highly rich in nutritious materials like-vitamins, minerals, enzymes, natural sugars, amino acids, and lots more essential nutrients. They are also highly antioxidant in nature. The leaves of *A. vera* are succulent, erect and form a dense rosette. The gel extracted from the leaves have innumerable applications and they are mainly cultivated worldwide as a crop for "*Aloe* gel". As per the report provided by the Kew Gardens, England's Royal Botanical Centre of Excellence, *A. vera* has been used for centuries and is currently more popular than ever (Lanka, 2018). It has been observed that the heterogenous compositions of *A. vera* may contribute to the diverse pharmacological and therapeutic activities. For different studies and researches it was proved that the *A. vera* gel can reduce tumor burden, tumor shrinkage, tumor necrosis and they are also well known to have chemo-preventive and anti-

genotoxic effects. Different studies on the *A. vera* gel claimed that the polysaccharides which are present in the *A. vera* gel extract is believed to have many therapeutic properties such as wound healing, anti-inflammatory effects, anti-diabetic, anti-microbial activities etc. (Alege and Ojomah 2014).

The extracted raw gel of *Aloe vera* plays an important role in the field of cosmetology and in food industry, and also it is one of the most essential and important natural ingredients which is being used in these industries from good old days. But the raw *A. vera* gel extract also possesses certain toxic and carcinogenic substances. Chemical analysis revealed that certain chemical substances such as polysaccharides, anthraquinones (Phenolic chemicals) are also found in the raw *A. vera* gel extract. So, ingestion of this raw *A. vera* gel extract without further processing can lead to certain diseases like diarrhea, hypokalemia, pseudomelanosis coli, kidney failure and may also lead to certain hypersensitive reaction (Guo and Mei 2016). In 2013, research conducted, clearly shows that the leaf extract of *A. vera* plant can cause carcinogenic activity in F344/N Rats (Bodreau *et al.,* 2013). Thus, from this we can conclude that though *A. vera* gel extract has many medicinal properties and values, but it also possesses certain cytotoxic and carcinogenic properties when they are used in raw form.

It is very much evident that colchicine acts as an inhibitor in mitotic cell division stages. It inhibits the formation of mitotic spindle which ultimately results in disruption in mitotic stages before cytokinesis. Therefore, the current scientific investigation has been designed to evaluate the effect of raw *Aloe vera* gel on cell division stages of *Allium cepa* and *A. sativum*. In this scientific investigation, root tips of *A. cepa* and *A. sativum* are taken as samples to know about the comparative study of the cytotoxic effects of raw *A. vera* leaf extract on both the root tips. Here the various concentrations of raw *A. vera* leaf extracts were prepared and then the root tips of both *A. cepa* and *A. sativum* were treated for about 24 hours. The main aim is to evaluate whether there are any damaged cells present in the slides prepared by the squashing method (Sinha *et al.,* 2019) and then to compare the cytotoxic effects on both the root tips. Thus, from this experiment we will come to know whether the fresh *A. vera* leaf extract possess any cytotoxic property or not. The root tips of *A. cepa* and *A. sativum* were used as a sample because the root tips of *A. cepa* can be grown easily in the room temperature. The *Allium* test has also been widely used to determine the cytotoxic and genotoxic effects as the results exhibited by these root tips are similar with the results exhibited by the mammalian test system (El-Shahaby *et al.,* 2003). The root tips of *A. sativum* have also been widely used to determine the cytotoxic and genotoxic effects because *A. sativum* contains few numbers of chromosomes and which are suitable for such cytological studies. It also presents a very clear illustration about the aberrations that occurs in the chromosomes and contains genetically uniform cloves (Alege and Ojomah 2014).

Bhalla *et al.* (1976) studied the cytological responses of root tip cells of *Allium sativum* to smoke puffs from various types of cigarettes. They found out that the mitotic abnormalities increase with the increasing number of puffs. Avila *et al.* (1997) observed that the cytotoxicity of low molecular weight fraction from *Aloe vera* gel and found out that *A. vera* gel contains toxic low molecular weight compounds. Langmead *et al.* (2004) observed the anti-inflammatory effects of *A. vera* gel in human colorectal mucosa in-vitro and found out that *A. vera* gel had dependent inhibitory effect on reactive oxygen metabolite procedure. And these provide support for the proposal that *A. vera* gel may have a therapeutic effect in inflammatory bowel disease. Saxena *et al.* (2005) did an experiment to know the cytogenetic effects of cypermethrin in root meristem cells of *Allium sativum.* They found out that the cells analyzed immediately after the exposure had a significant, dose-dependent inhibition of mitotic index (MI) and they also observed mitotic induction and chromosomal aberrations. Boudreau and Beland (2006) evaluated the biological and toxicological properties of *Aloe barbadensis* (Miller), *Aloe vera*. In their experiment they found out that the ingestion of *A. vera* is associated with certain diseases like- diarrhea, electrolyte imbalance, kidney dysfunction and conventional drug interactions. Gul *et al.* (2006) did a study on the genotoxic effects of avenoxan on *Allium cepa* and *A. sativum* at different concentrations and different time period. They observed that at all the concentrations of avenoxan shows induced abnormalities as well as a decrease in the mitotic index. They got similar results in both *A. cepa* and *A. sativum.* Dongli *et al.* (2009) studied the effect of colchicine on the mitotic division of root tip cells of *Allium sativum.* They observed that the mitotic index decreased at the increasing time duration and concentration of colchicine. The cytogenetic toxicity of the crude leaf extract of *Aloe vera*, medicinal plant, was evaluated in two test system: onion and Swiss albino mice, by using their root tip meristematic cells and bone marrow cells respectively. They observed a marked increase in cells with chromosome number anomalies and a significant increase in the mitotic index of both cell types (Verma *et al.,* 2012). Ilbas *et al.* (2012) observed the effect of *A. vera* leaf extract on the root tip cells of *Allium cepa* L. and found that the mitotic index and root growth rate of *A. cepa* were considerably decreased in comparison to the control *A. cepa*. Cytotoxic effects of *A. vera* leave gel extract was carried out on mice bone marrow cells. The results showed significant effect of *A. vera* extract for both doses on mitotic index (MI) and chromosomal aberration (Abdelrawaf *et. al.,* 2013). These findings also agree with the observations done by Kayraldiz *et al.* (2010), where they observed a decrease in mitotic index

in bone marrow cells of rats. Boudreau *et al.* (2013) observed the toxicological and carcinogenesis studies of a non-decolorized whole leaf extract of *A. barbadensis* Miller (*A. vera*) in F344/N rat and B6C3F1 mice. They found that in all groups of rats and mice treated with *A. vera* extract the rates of hyperpalasia in the large intestine were markedly increased compared to the normal ones. Alege and Ojomah in (2014) found out that at different levels of concentration the leaf extract of *A. vera* have cytotoxic effects on the mitotic cell division on the root tip cells of *Allium sativum.* The result revealed that the addition of *A. vera* gel extract affects the mitotic activities of the root tip cells of *A. sativum.* Cavusoglu *et al.* (2016) found out that the application of *A. vera* L. leaf extract on *Allium cepa* seeds (germinated under salt stress) induces chromosomal aberrations and micronucleus formation, which indicates genotoxic and had cytotoxic activities in normal conditions. Guo and Mei (2016) did a chemical analysis of the *Aloe vera* plant and found out that it contains various polysaccharides and phenolic chemical, notably anthraquinones. They also found out that ingestion of Aloe preparations is associated with diarrhea, hypokalemia, pseudomelanosis coli, kidney failure, as well as phytotoxicity and hypersensitive reactions. Benzidia *et al.* (2019) carried out a study on the chemical composition and antioxidant activity of tannins extract from green rind of *A. vera* by the gas chromatography process. They found that the tannins extracted from the *A. vera* shoed some antiradical activity. Babu and Noor (2020) wrote a review paper on the bioactive constituents of the genus Aloe and their potential therapeutic and pharmacological applications. In this paper the author mentioned that the various bioactive constituents obtained from the genus Aloe can be used to treat various problems. So, they suggested the need for further research on the genus Aloe for the improvement of health care. Khan *et al.* (2020) carried out a study on the genotoxic effect of two commonly used food dyes metanil yellow and carmoisine by using *Allium cepa* L. as the indicator. During this study they observed that both the dyes showed reduction in mitotic index. They have also observed various chromosomal aberrations like- disorientation at metaphase, metaphase stickiness, c-mitosis, anaphase bridge etc. Thus, the authors suggested that though the food dyes have been used frequently, so it is very much essential to use these dyes in limited and controlled dose. Shilpa *et al.* (2020) carried out a study on the antifungal activity of *Aloe vera* Leaf and gel extracts against *Candida* albicans. Thus, by comparing the results obtained the authors concluded that the ethanolic extract of *A. vera* gel possesses considerable antifungal activity against *C. albicans*. Akinboro and Jimoh (2021) carried out a study on antigenotoxic potential of gel extract of *Aloe vera* against sodium azide genotoxicity in *Allium cepa* cells. Thus, by comparing the results the authors concluded that at lower concentration the *A. vera* gel extract can be used to develop many anticancer drugs and it can also reduce the toxicity of sodium azide at low concentration, but at higher concentration the *A. vera* gel itself becomes toxic. Alven *et al.* (2021) wrote a review paper on the role of *Aloe vera* extract for the treatment of wounds. In this paper the authors mainly discussed about the facts that if plant extracts can be added to the polymer-based dressings then it can enhance its biological properties like- antibacterial, anti-inflammatory etc. Babu *et al.* (2021) carried out a study on the role of zonulin and GLP-1/DPP-IV in the alleviation of diabetes by peptide/polypeptide fraction (PPF). During this study the authors have found that as PPF reduces fasting plasma glucose level then it led to the increase in insulin levels in diabetic rate. On the other hand, they have also observed an inverse relation between GLP-1 and DPP-IV. Barman *et al.* (2021) carried out a study on the mitotic abnormality inducing effects of aqueous leaf extract of *Clerodendrum inerme* Gaertn. on the root tip cells of *Allium cepa*. During this study the authors observed similar mitotic abnormalities in the root tip cells treated with both colchicine and *C. inerma*. Thus, the author suggested the need for further study of the bioactive substances of *C. inerma*. Gulati (2021) wrote a review paper on the medicinal properties of the *Aloe vera* plant. In this paper the author has mentioned 14 different medicinal properties provided by the *A. vera* plant. They have also mentioned about the various bioactive components present in the *A. vera* gel. Mahabady *et al.* (2021) carried out a study on the effect of *Aloe vera* on the expression of two nerve factors p75 and TrkA receptors in the hippocampus of diabetic rats. By comparing the results, the authors concluded that the addition of *A. vera* gel in rats having diabetes reduces diabetes induced hyperglycemia and it also reduces the expression of NGF and p75. On the other hand, there was an increase in the expression of TrkA receptor. Yadav and Kumar (2021) carried out a study on the assessment of cytotoxic potentiality of aqueous gel solution of *Aloe barbadensis* on the root tip cells of *Trigonella foenum-graecum* L. During this study the authors observed a dose dependent reduction of Mitotic Index (MI) and various chromosomal aberrations have also been observed. Al-Ghazali (2022) carried out a study on the anti-candida activity and cytotoxic effects of *Aloe vera* leaf extract against melanoma and normal cell lines. Thus, by comparing the results obtained the author suggested that the need to purify and identify the various bioactive compounds in the *A. vera.* Amin and Ibrahim (2022) carried out a study on the modulation of cytotoxic effects of wastewater on barely seedlings by *Aloe vera* extract. Thus, they comparing the result the authors concluded that the *A. vera* increased the mitotic index whereas the wastewater reduces the mitotic index. Barman and Ray (2022) carried out a study on the cytotoxic effects of leaf aqueous extract of *Maesa*

macrophylla in *Allium cepa* root tip cells. Thus, by comparing the results, the authors concluded that there was a dose and time period dependent enhancement in root growth retardation (RGR) and a dose dependent reduction of mitotic index in the root tip cells. Jangra *et al.* (2022) wrote a review paper on the dark side of *A. vera* plant. In their paper they have mentioned that the *A. vera* plant contains several active compounds. So, it is a matter of concern and there is a need to develop some mechanism to understand the toxic compounds. Velazquez-Vazquez *et al.* (2022) carried out a study on the genotoxicity and cytotoxicity of *Sambucus canadensis* ethanol extract on the root tip cells of *Allium sativum.* During this study they observe a dose dependent reduction in the number of cells in division phase and observed various cellular abnormalities in the root tip cells of *Allium sativum.* Pharmawati and Wrasiati (2023) carried out a study on the chromosomal and nuclear alteration induced by nickel nitrate in the root tip cells of *Allium cepa* var. aggregatum. By comparing the results, the authors concluded that since the element Ni at its lowest concentration with the exposure period of 72 hours also showed genotoxic effects on the root tip cells of *A. cepa.*

MATERIALS AND METHODS

Collection of Materials. Fresh bulbs of onions and garlics were purchased from the nearby market (Six Mile market, Guwahati) and they were allowed to grow in normal tap water in a 100ml of conical flask at room temperature near a window so that they get fresh air and sunlight. *Aloe vera* plants were bought from the market and they were grown in a tub at home.

Preparation of test materials. As the leave reaches a height of about 15-20 cm, they were cut and washed thoroughly. Now from these washed leaves fresh gel was extracted and grinded in a blender with a small amount of water so that the roots of both onion and garlic could be able to absorb it. After that different concentration (20%, 40%, and 60%) of *A. vera* gel extract was prepared for performing the experiment by adding the required amount of water, i.e., for making 20% of *A. vera* gel extract 20% of gel and 80% of water is required and so on for other concentrations.

Procedure. Once the onion and garlic roots reached a length of about 3-5cm, they were transferred into the *Aloe vera* gel and were allowed to remain there for 24 hrs. i.e., the onion and garlic roots were treated with different concentrations of *A. vera* gel extract with the time duration of 24 hours. And now the remaining procedure of squashing the root tips and making microscopic slides were done by following the protocols mentioned in the practical book named "Advanced Practical Zoology" (Sinha *et al.,* 2019). After making the slides they were observed under a microscope to observe if any difference is found in the slides prepared from the root tips treated with *A. vera* gel extract.

Data Analysis. In this scientific investigation for the purpose of result twenty different counts were taken for total number of cells, number of dividing cells and number of affected cells for all the three different concentrations of *Aloe vera* gel extracts i.e., 20%, 40% and 60%. Statistical analysis was done through "Statistical Calculator- Calculator Soup". The statistical data were subjected to one-way Analysis of Variance (ANOVA). The mitotic index (MI) for the cells treated with different concentration of *Aloe vera* gel extract was calculated using the formula proposed (Auti *et al.,* 2010) as given below

Mitotic Index,

$$MI = \frac{\text{Total number of dividing cells}}{\text{Total number of cells examined}} \times 100$$

Microscopic Observation. The prepared slides were observed through a stereo Olympus trinocular microscope (Model No.: CH20i). The 4x, 10x and 40x objectives were used for viewing the slides. Photographs of different observed slides were taken using ONEPLUS 6 mobile with android version 10 (Model: ONEPLUS A6000).

RESULTS AND DISCUSSION

Descriptive Statistics. The descriptive statistics for different concentrations of *Aloe vera* treatment on onion (*Allium cepa)* root tip cells are shown in (Table 1). The result shows that in 20% of *A. vera* gel extract the mean is 43.6, in 40% gel extract the mean is 254.65 and in 60% gel extract the mean is 370.3. Likewise, the maximum value in 20% extract is 40 and the minimum value is 47. In 40% extract the maximum value is 259 and the minimum value is 250, while in 60% extract the maximum value is 374 and the minimum value is 367. Similarly, the standard deviation in 20% extract is 2.233713, in 40% extract the standard deviation is 2.3457689 and the standard deviation in 60% is 2.05452. The descriptive statistics for different concentrations of *Aloe vera* treatment on garlic (*Allium sativum*) root tip cells are shown in (Table 2). The result shows that in 20% *A. vera* gel extract the mean is 29.65, in 40% extract the mean is 228.05 and in 60% extract the mean is 324.60. Likewise, the maximum value in 20% extract is 33 and the minimum value is 26, in 40% extract the maximum value is 232 and the minimum value is 235 and in 60% extract the maximum value is 329 and the minimum value is 320. Similarly, the standard deviation in 20% is 2.3457689, in 40% the standard deviation is 2.2354795 and in 40% extract the standard deviation is 2.5214866.

Table 1: Descriptive statistics of *Aloe vera* treatment on Onion root tip cells.

		20%	40%	60%
Minimum	min =	40	250	367
Maximum	max =	47	259	374
Range	R =	7	9	7
Count	n =	20	20	20
Mean	$\bar{x}$ =	43.6	254.65	370.3
Median	$\tilde{x}$ =	43.5	255	370
Mode	mode =	43, 42, 46, 45	256	370
Standard Deviation	s =	2.233713	2.3457689	2.05452
Variance	s^2 =	4.9894737	5.5026316	4.2210526
Sum	sum =	872	5093	7406

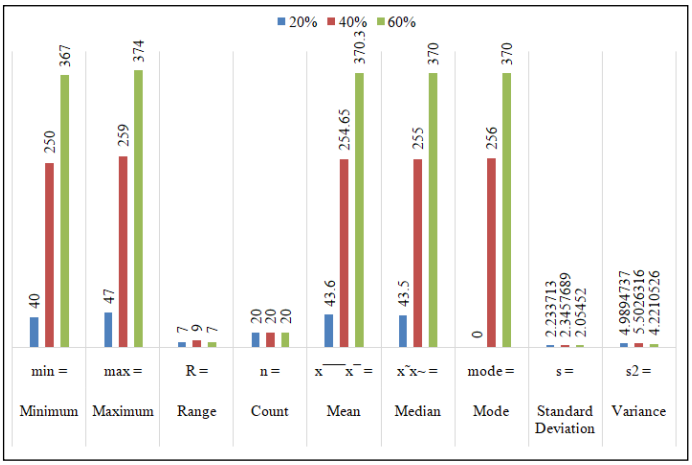

Fig. 1. Graphical representation of Descriptive Statistics for different concentration of *Aloe vera* treatment on onion root tip cells.

Table 2: Descriptive statistics of *Aloe vera* treatment on Garlic root tip cells

		20%	40%	60%
Minimum	min =	26	225	320
Maximum	max =	33	232	329
Range	R =	7	7	9
Count	n =	20	20	20
Mean	$\bar{x}$ =	29.65	228.05	324.6
Median	$\tilde{x}$ =	30	227.5	325
Mode	mode =	27, 30, 31, 33	230, 227	326
Standard Deviation	s =	2.3457689	2.2354795	2.5214866
Variance	s2 =	5.5026316	4.9973684	6.3578947

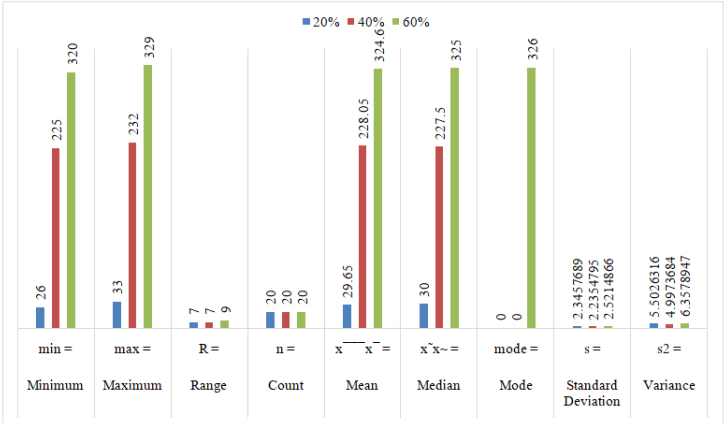

Fig. 2. Graphical representation of Descriptive Statistics for different concentration of *Aloe vera* on garlic root tip cells.

Again, from this study it is observed that the mitotic index (MI) of *Allium cepa* root tip cells decrease with the increasing concentrations of *Aloe vera* gel extract, i.e., in 20% of *A. vera* gel extract the MI is 32.68, while in 40% extract the MI is 28.2 and in 60% extract the MI is 21.02. Similarly, in case of *A. sativum* the MI also decreases with the increasing concentrations of *A. vera* gel extract, i.e., in 20% extract the MI is 66.98, while in 40% extract the MI is 50.58and in 60% extract the MI is 45.21. Likewise, the rate of affected cells in case of *A. cepa* in 20% gel extract is 1.33, in 40% extract it is 8.07 and in 60% extract it is 12.01, while the rate of affected cells in case of *A. sativum* in 20% extract is 1.2, in 40% extract it is 8.6 and in 60% extract it is 13.18.

Table 3: Mitotic Index (MI) and the rate of affected cells.

	20%		40%		60%	
	ONION	GARLIC	ONION	GARLIC	ONION	GARLIC
Mitotic Index	32.68 ± 0.79	66.98 ± 0.13	28.2 ± 0.19	50.58 ± 0.15	21.02 ± 0.18	45.21 ± 0.12
Rate of Affected Cells	1.33 ± 0.06	1.2 ± 0.09	8.07 ± 0.10	8.6 ± 0.08	12.01 ± 0.06	13.18 ± 0.09

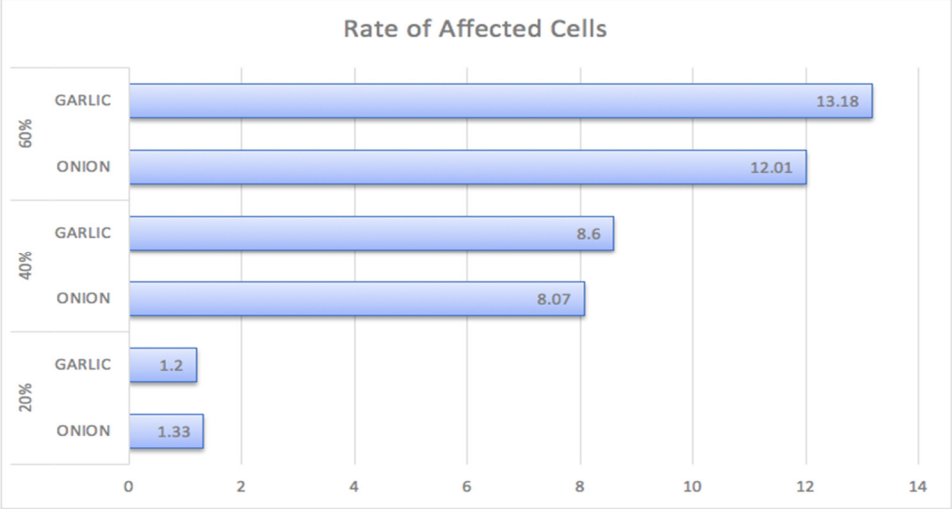

Fig. 3. Graphical representation of the Rate of Affected Cells of Onion and Garlic.

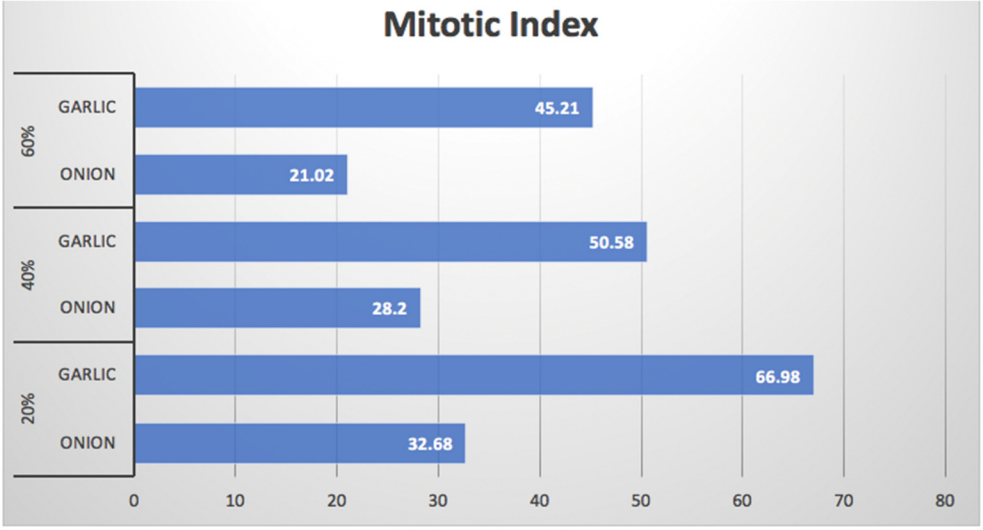

Fig. 4. Graphical Representation of Mitotic Index (MI) of Onion and Garlic.

Table 4: The affected cell mean ration.

Root mean Square	20%		40%		60%	
	ONION	GARLIC	ONION	GARLIC	ONION	GARLIC
	43.60 ± 0.23	29.65 ± 0.12	254.50 ± 0.08	228.05 ± 0.21	370.30 ± 0.19	324.60 ± 0.24

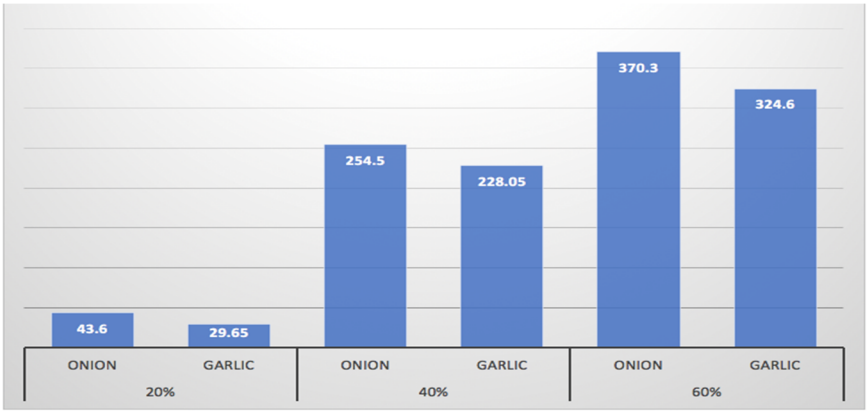

Fig. 5. Graphical representation of the Affected cells Mean ratio.

The Analysis of Variance (ANOVA) results for the onion (*Allium cepa*) root tip cells treated with the increasing concentrations of *Aloe vera* gel extract are shown in Table 5. The result shows that the Degree of Freedom (DF) between groups is 2 and the DF within groups is 57. Likewise, the sum of squares (SS) between groups is 1097475.6 and the SS within groups is 361.9958. Similarly, the mean square (MS) value between groups is 548737.8 and the MS value within group is 6.3508. The F-statistic value between groups is 86404.4716 and the P- value is <0.00001.

Table 5: Analysis of Variance (ANOVA) Results (ONION).

Data Summary					
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
20% conc.	20	43.6	2.2337	0.4995	
40% conc.	20	254.5	3.1372	0.7015	
60% conc.	20	370.3	2.0545	0.4594	

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	2	1097475.6	548737.8	86404.4716	<0.00001
Within Groups	57	361.9958	6.3508		
Total:	59	1097837.5958			

F-statistic value = 86404.47161; P-value = <0.00001

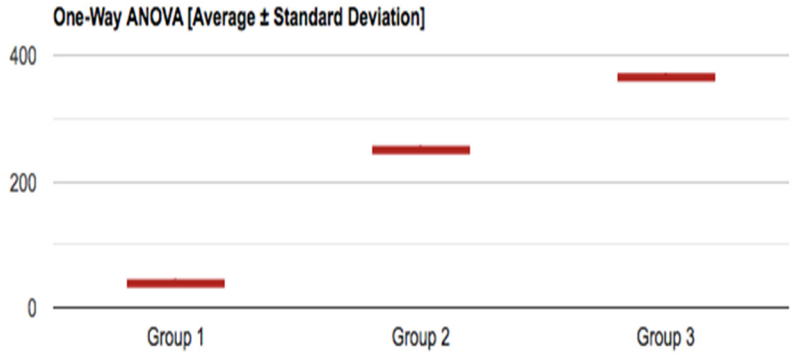

Fig. 6. Graphical representation of One-Way ANOVA (Average ± Standard Deviation of Onion.

The Analysis of Variance (ANOVA) results for the garlic (*Allium sativum*) root tip cells treated with increasing concentrations of *Aloe vera* gel extract are shown in (Table 6). The result shows that the Degree of Freedom (DF) between groups is 2 and the DF within groups is 57. Likewise, the sum of squares (SS) between groups is 904533.2 and the SS within groups is 320.3058. Similarly, the mean square (MS) value between groups is 452266.55 and the mean square (MS) value within groups is 5.6194. The F-statistic value between groups is 80483.0669 and the P-value between groups is <0.00001.

Table 6: Analysis of Variance (ANOVA) Results (GARLIC).

Data Summary					
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
20% conc.	20	29.65	2.3458	0.5245	
40% conc.	20	228.05	2.2355	0.4999	
60% conc.	20	324.6	2.5215	0.5638	

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	2	904533.1	452266.55	80483.0669	<0.00001
Within Groups	57	320.3058	5.6194		
Total:	59	904853.4058			

F-statistic value = 80483.06692; P-value = <0.00001

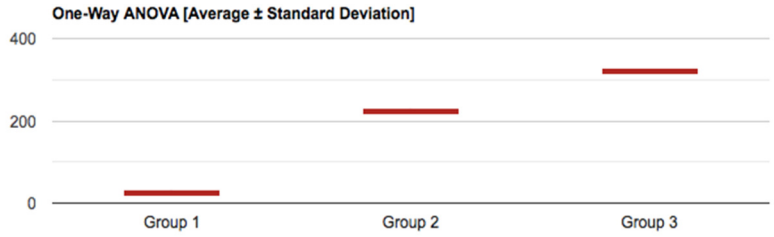

Fig. 7. Graphical representation of One-Way ANOVA (Average ± Standard Deviation) of Garlic.

Table 7: Analysis of Variance Results (Between Onion & Garlic 20%)

Data Summary					
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
ONION	20	43.6	2.2337	0.4995	
GARLIC	20	29.65	2.3458	0.5245	

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	1	1946.025	1946.025	370.9472	<0.00001
Within Groups	38	199.3517	5.2461		
Total:	39	2145.3767			

F-statistic value = 370.94723; P-value = <0.00001

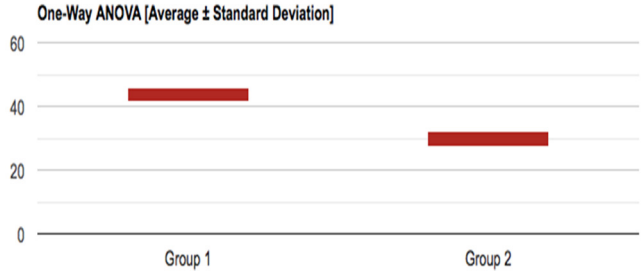

Fig. 8. Graphical representation of One-Way ANOVA (Average ± Standard Deviation) between Onion and Garlic 20%

Table 8: Analysis of Variance (ANOVA) Results (Between Onion & Garlic 40%).

Data Summary					
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
Group 1	20	254.5	3.1372	0.7015	
Group 2	20	228.05	2.2355	0.4999	

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	1	6996.025	6996.025	942.8933	<0.00001
Within Groups	38	281.9502	7.4197		
Total:	39	7277.9752			

F-statistic value = 942.89329; P-value = <0.00001

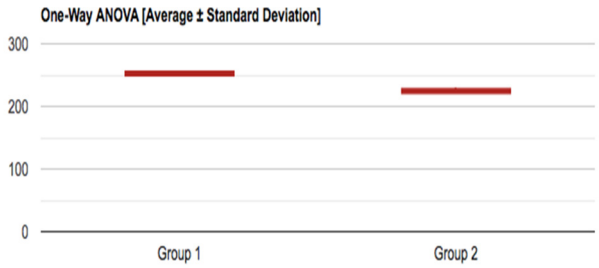

Fig. 9. Graphical representation of One-Way ANOVA (Average ± Standard Deviation) between Onion and Garlic 40%.

Table 9: Analysis of Variance (ANOVA) Results (Between Onion & Garlic 60%).

Data Summary					
Groups	**N**	**Mean**	**Std. Dev.**	**Std. Error**	
Group 1	20	370.3	2.0545	0.4594	
Group 2	20	324.6	2.5215	0.5638	

ANOVA Summary					
Source	**Degrees of Freedom DF**	**Sum of Squares SS**	**Mean Square MS**	**F-Stat**	**P-Value**
Between Groups	1	20884.9	20884.9	3948.3946	<0.00001
Within Groups	38	200.9997	5.2895		
Total:	39	21085.8997			

F-statistic value = 3948.3946; P-value = <0.00001

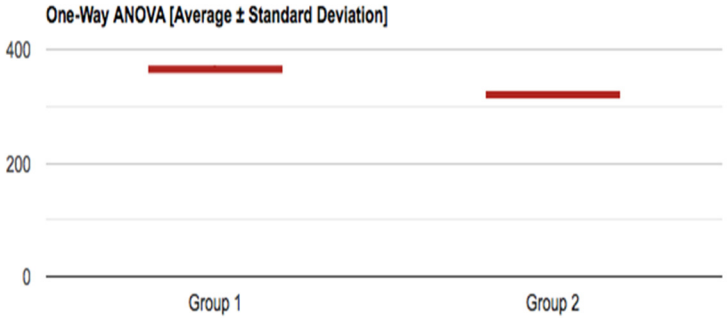

Fig. 10. Graphical representation of One-Way ANOVA (Average ± Standard Deviation) between Onion and Garlic 60%.

SUMMARY OF OUR EXPERIMENT

In this study when the results between garlic and onion root tip cells in 20%, 40% and 60% *Aloe vera* gel extract was summarized using Analysis of Variance (ANOVA), it was observed that the effect of 20% of *A. vera* extract for the exposure of 24 hours on onion is higher than the garlic (Table 7). Likewise, the effect of 40% extract for the exposure of 24 hours is also higher than the garlic (Table 8). Similarly, the effect of 60% extract for the exposure of 24 hours is also higher than the garlic (Table 9). Thus, by comparing the tables we can conclude that the effect of raw *Aloe vera* gel extract is higher in onion root tip cells than garlic root tip cells. Thus, we can say that onion is very much susceptible than garlic. In this study *Allium cepa* and *A. sativum* are used because they can be grown easily in the room temperature and they can also give clear illustration about the observation in the chromosomes and they also showed similarity with the results exhibited by the mammalian test system which was performed by El-Shahaby *et al.* (2003).

Alege and Ojomah, 2014 did an investigation on the cytotoxic effects of *Aloe vera* leaf extract on *Allium sativum* root tips by Analysis of Variance (ANOVA) and found out the mitotic index (MI) of the roots treated with concentrations of 0%, 25%, 50%, 75% and 100% *A. vera* extracts for 24 hours are 2.79%, 40.01%, 38.76%, 36.38% and 36.37%. Thus, in this scientific investigation they concluded that the mitotic index reduces with increasing concentrations of *A. vera* extract applied on the root tip cells of *A. cepa*. Ilbas *et al.* (2012) did an investigation on the cytotoxicity of *A. vera* gel extracts on *A. cepa* root tip cells by one-way Analysis of Variance and Ducan's multiple range tests and found out that the MI of the root tip cells treated with concentrations of 0%, 2%, 5%, 10%, 20% and 40% *A. vera* extracts for 24 hours are 9.29, 9.31, 8.74, 7.97, 6.68 and 4.14 respectively and for 48 hours the MI are 9.07, 8.88, 8.82, 7.09, 6.54 and 3.72 respectively. Thus, they concluded that the cytotoxic effect of *A. vera* gel extract depends on their concentration rather than the time

period. Shilpa *et al.* (2020) carried out a study on the antifungal activity of *Aloe* vera leaf and gel extract against *Candida albicans* invitro by well diffusion method. From this study they have observed that at 500μL concentration the inhibition % was 94.2% and growth % was around 5.8%, while at 400μL concentration the inhibition % was around 99.33% and growth % was 0.67. On the other hand, at 300μL concentration the inhibition was around 94.2% and growth % was around 5.8% and in 200μL concentration the inhibition % was around 98.2% and growth % was around 1.2%. Verma *et al.* (2012) did an investigation on the cytogenetic toxicity of *A. vera* on onion root tip cells and bone marrow cells of Swiss albino mice. They found out that at 0.5% concentration of *A. vera* gel extract the MI for onion root tip cell is 95.56 for 24 hours and at 1 % extract the MI of onion root tip cell is 98.03 for 24 hours. On the other hand, the mitotic index of bone marrow of Swiss-albino mice T_1 group (1g of *A. vera* extract/kg body weight + 4mg of colchicine/kg body weight) is 65.42 and the MI of mice T_2 group (2g of *A. vera* extract/kg body weight + 4mg of colchicine/kg body weight) is 73.48. Thus, they concluded that the extract significantly increases the mitotic index on both cell types. Al-Ghazali (2022) carried out a study on the anti-candida activity and cytotoxic effects of the *Aloe* vera leaf extract against melanoma and normal cell lines. The author concluded that the *A. vera* gel possesses a strong anticancer and anti-tumor property and it can be used as an alternative drug in cancer therapy.

Alven *et al.* (2021) focuses on the therapeutic outcomes of *A. vera* extract -loaded polymer-based scaffolds in wound management. Babu *et al.* (2021) carried out a study on the role of zonulin and GLP-1/DPP-IV in alleviation of diabetes by peptide/polypeptide fraction (PPF) of *Aloe vera* in streptozotocin-induced diabetic wistar rats. During this study they have found that as PPF reduced fasting plasma glucose level, it leads to the increase in insulin level in streptozotocin-induced diabetic rats. They have also found an inverse relation between GLP-1 and DPP-IV. Cavusoglu *et al.* (2016) did an investigation on the effects of *A. vera* leaf extract on some physiological and cytogenetical parameters in *A. cepa* L. seed germinated under salt stress and they found out that the MI in Group-I, i.e., control is 5.6 and the MI of Group-II, i.e., treated with 0.15 M NaCl, for seven consecutive days is 3.7. Similarly, in Group III, i.e., treated with 0.1mg/L dose of *A. vera* leaf extract for seven consecutive days the MI is 16.5 and in Group IV, i.e., treated with 0.1mg/L dose of *A. vera* leaf extract and 0.15 M NaCl for seven consecutive days the MI is 24.6. Thus, they concluded that the root tip meristem of *A. cepa* germinated in the media containing 0.15 M NaCl decreased 66% as compared with the Group I seed.

Abdelrawaf *et al.* (2013) did an investigation on the cytogenetic effects of *A. vera* leaves gel extract on mice bone marrow cells through one-way Analysis of Variance (ANOVA) and they found out that the MI in groups treated with 40mg/kg body weight of mice (BW) is 3.44, while with 80mg/kg BW the MI is 2.0153. Since, the MI decreases with the increasing concentration of the *A. vera*, so by this study they concluded that the *A. vera* gel have some significant effect on the bone marrow cells. Kayraldiz *et al.* (2010) did an experiment on the effect of *A. vera* leaf extract and ethyl carbonate (EC) on the bone marrow cells of rats and they found out that the MI at the exposure time period of 12 hours is different, i.e., in the control group the MI is 5.77, the MI in the EC (400mg/kg) is 3.68, the MI in *A. vera* (AV) 750mg/kg is 5.36, while, the MI in AV 1000mg/kg is 5.29 and the MI in AV 1250mg/kg is 4.59. When both AV & EC are mixed then the MI at 750 AV+EC400 is 3.72, while at 1000AV+EC400 the MI is 34.8 and at 1250AV+EC400 the MI is 3.17. They repeated the same experiment but at the exposure of 24 hours and they found the following MI: at EC 400mg/kg the MI is 3.81, at AV 700mg/kg the MI is 3.74, at AV 1000mg/kg the MI is 3.70, at AV 1250 mg/kg the MI is 3.63, at 750AV+400EC the MI is 3.66, at 1000AV+400EC the MI is 3.40 and at 1250AV+400EC the MI is 3.20. Thus, they concluded that in the bone marrow cells of rat *A. vera* leaf extract induces structural and chromosomal abnormalities at all concentrations in all the treatment period and the mixture of AV and EC also induces structural and chromosomal abnormalities at the concentrations and time period. But the effect of the mixture of AV and EC is higher than AV and EC alone.

Gul *et al.* (2006) did an investigation on the genotoxic effects of Avenoxan on the root tip cells of *A. cepa* and *A. sativum* and they found out that the MI decreases with increasing concentration of Avenoxan in both *A. cepa* and *A. sativum*. The complete inhibition was seen at 12 hours treatment period. Thus, they concluded that the inhibition of MI was completely dependent on the concentration and time period of treatment. In their scientific investigation they also mentioned that they obtain similar results in both *Allium cepa* L. and *A. sativum.* Amin and Ibrahim (2022) carried out a study on the modulation of cytotoxic effects of wastewater on barely seedlings by *Alo vera* extract. The addition of *A. vera* extract increased the accumulation of protein in both shoot and root. While, the alcoholic extract of *A. vera* gel also promotes increase in both root and shoot protein o. Morepver, the *A. vera* extract also increased the mitotic index, while the wastewater reduced the mitotic index. Yadav and Kumar (2021) carried out a study on the assessment of cytotoxic potentiality of aqueous solution of *Aloe barbadensis* (AB) on the root meristem cells of *Trigonella foenum-graecum* L. A dose dependent reduction in the maximum mitotic index (MI) was observed at 100ppm concentration for the exposure period of 2 hours, which was around 18.05±2.13. On the other hand, the minimum MI was observed at 1000 ppm concentration for the exposure period of 6 hours, which

was around 11.11±1.72. Moreover, the various types of aberrations observed during this study were- breakage of chromosomes at metaphase, disturbed metaphase, chromosomal extrusion at metaphase, stickiness of chromosomes, polyploidy at metaphase, scattered metaphase etc. Pandir (2018) did an investigation on the genotoxic effect of diazinon on root tip cells of A. cepa and they found out that the MI is highest at control group at the time exposure of 24 hours and the MI is lowest at 160ppm concentration at the time exposure of 72 hours, they observed that the MI decreases as the concentration and the time of exposure increases. Thus, they concluded that diazinon have some inhibitory effect on the root tip cells of A. cepa. Mangalampalli *et al.* (2018) did an investigation on the toxic activity of magnesium oxide nanoparticels (NP) and microparticles (MP) and they found out that at different concentrations of NP the MI is also different, i.e., at 12.5µg/mL concentration the MI is 43.6, while at 25µg/mL concentration the MI is 42.6, similarly at 50µg/mL concentration the MI is 39.0 and at 100µg/mL concentration the MI is 37.6. Likewise at different concentrations of MP the MI is also different, i.e., at 12.5µg/mL concentration the MI is 43.5, at 25µg/mL concentration the MI is 40.3, similarly at 50µg/mL concentration the MI is 41.0, while at 100µg/mL concentration the MI is 41.0 and at the control group the MI is 45.9.

Benzidia *et al.* (2019) did an investigation on the chemical composition and antioxidant activity of tannin extract from green rind of *A. vera* by gas chromatography. During their study they found four main constituents – Palmitic acid (11.91%), E-Phytol (14.40%), Linolenic acid (16.59%), Diisooctylphtalate (11.84%). They also fractioned the tannin extract over a silica gel dry column and isolated three main fractions. 25.99% of Palmitic acid was recorded in the first fraction, while 30.93% of Dibutyl phthalate was obtained in the second fraction and in the third fraction 54.13% of Diisooctylphtalate was recorded. The tannin extracts also showed a mild presence of antiradical activity. Barman *et al.* (2021) carried out a study on the mitotic abnormality inducing effects of aqueous leaf extract of *Clerodendrum inerme* Gaertn. On the root tip cells of *Allium cepa*. the various mitotic abnormalities induced by *A. inerme* were- Sticky chromosomes, polar deviation, anaphase bridge, vagrant chromosome, lagging chromosome, ring chromosome, multipolar anaphase, and multipolar telophase. On the other hand, various mitotic abnormalities induced by colchicine were- Sticky chromosome, polar deviation, anaphase bridge, vagrant chromosome, lagging chromosome, ring chromosome, multipolar anaphase, and multipolar telophase. Velazquez -Vazquez *et al.* (2022) carried out a study on the genotoxicity of *Aambucus canadensis* ethanol extract on the root tip cells of *Allium sativum.* The number of cell in division was maximum at 125mg/L concentration, which was around 1980, while it was minimum at 1500mg/L concentration, which was around 602. The various cellular abnormalities observed during this study were- Bridges, chromosome fragemnts, binucleate, chromosome lagging and disoriented, sticky chromosome, vagrant chromosome etc. Jangra *et al.* (2022) wrote a review paper on the dark side of the *A. vera* plant. During their study they have mentioned that although the plant is known to have various medicinal values and can cure many diseases, but it also possesses certain toxic substances which can cause various diseases and abnormalities. In their paper they have also mentioned that *A. vera* plant can reduce the synthesis of prostaglandin, which ultimately inhibits the secondary aggregation of platelets at the wound site. Consumption of *A. vera* can also cause diseases like- diarrhea, kidney failure, toxic hepatitis etc. So, they suggested that though the plant has several benefits, but along with that it also has many side effects, so everyone should consider both the benefits as well as the side effects of the plant. Pharmawati and Wrasiati (2023) carried out a study on the chromosomal and nuclear alteration induced by nickel (Ni) nitrate in the root tip cells of *Allium cepa* var. aggregatum.

CONCLUSIONS

The result of this study indicates that the raw *Aloe vera* gel extract exhibits some cytogenetic effect on both the root tip cells of onion (*Allium cepa*) and garlic (*A. sativum*), but the effect of raw *A. vera* gel extract on the root tip cells of onion is much higher than the root tip cells. Thus, by comparing the results obtained during this scientific investigation we can conclude that the effect of raw *A. vera* gel extract is higher in onion root tip cells than garlic root tip cells. Thus, we can say that onion is very much susceptible than garlic. From this scientific investigation we can also conclude that the raw *A. vera* gel should not be consumed as it causes mitotic inhibition in both garlic and onion root tip cells. *A. vera* gel also causes various mutagenic effects in some animals like in rats as mentioned by various researchers. So, the *A. vera* gel should be consumed after processing and not as raw product.

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Conflict of Interest. None.

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