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Cytotoxic effect of Van Tulsi (Ocimum gratissimum) leaf extract on root tip cells of Onion (Allium cepa).

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Abstract: *The present work is designed to evaluate the cytotoxic effects of Van Tulsi (Ocimum gratissimum) leaf extract at two different durations (72 hrs and 96 hrs) on onion root tip cells. In 72 hrs treatment duration mitotic index slightly increased but at 96 hrs duration mitotic index significantly decreased due to decrease in the proportion of prophase and Metaphase cell population. Thus, leaf extract of Van Tulsi act as mitotic inhibitor on onion root tip cells at higher treatment duration.*

Keywords: *Mitotic index, Allium cepa, Cytotoxic, Ocimum gratissimum.*

1. INTRODUCTION:

Ocimum gratissimum, the medicinal herbs called as Van Tulsi have been used in folk medicine for million years. Simply, in recent times, scientific study of their effects has flourished. The use of species of the Ocimum genus in organic agriculture is described in different works especially in the culture of vegetables due to its bactericidal, antihelminthic [1].

Different parts including the leaves, stem, flowers and roots of Van Tulsi can be used. The leaves and stem are used in Chinese medicine for digestive system disorders, diarrhea and kidney infections, cough, especially chest colds, headache, and various types of fever [2].

Furthermore, over the last decade, medicinal plants and their bioactive compounds have attracted the attention of several researchers because of their usefulness in the management and prevention of life-threatening and chronic diseases such as cancer, diabetes, stroke and arthritis [3], as an alternative therapy for the treatment of psychiatric disorders [4]. This plant possesses numerous pharmacological properties such as aet anti-hyperglycaemic [5], [6], anti-inflammatory [7], [8], anti-diarrhoeal [9], anti-anaemic, hepatoprotective [10], anti-hypertensive [11], antibacterial [12], [13], hypoglycaemic [14], [6] antifungal [15] nematocidal [16], insecticidal [17], antimicrobial [14], fungicidal [18] and anti-oxidative properties [19], [20] as well as exhibits many other pharmacological activities.

The present study was carried out to evaluate the cytotoxic effect of ***Van Tulsi (Ocimum gratissimum) leaf extract on root tip cells of Onion (Allium cepa).***

2. MATERIALS AND METHODS:

PREPARATION OF LEAF EXTRACT: The leaves of Van Tulsi *(Ocimum gratissimum)* were washed under running tap water and shade dried for 2 to 3 weeks. After that/dried fresh Tulsi leaves were homogenised by using a grinder to made fine powder so obtained and stored in air tight bottles. 100gram fine powder were dissolved in 1000 ml distilled water as a stock solution and left for 48 hours. It was then filtered through Whatman No. 1 [21]. Van Tulsi leaf extract 5% dose was prepared by dilution of stock solution [22].

The onion bulb weighing approximately 20-30 grams were purchased from local market and their roots were initially allowed to grow till 1.5 cm in length in normal tap water. The bulb roots were cut after 72 hrs and 96 hrs and fixed in aceto-alcohol for 24 hrs then preserved in 70% ethanol and used as control group.

Another set of onion bulbs (20-30gm) were grown in 5% Van Tulsi leaf extract for 72 hrs and 96 hrs respectively and used as treated group.

Figure 1: (Growing of Control and Treated Onion root tips).

SLIDE PREPARATION:

After treatment, slides were prepared by Acetocarmine squash preparation [23]. Approximately 4000 cells were randomly analyzed in both control and treated group of onion bulbs. Frequency of Mitotic index and Phases distribution were calculated.

SLIDE SCREENING:

All the slides were examined under light microscope. The mitotic index was calculated for determination of cytotoxicity. Mitotic index (MI) was calculated as the ratio between the number of mitotic cells and the total number of cells scored and expressed as percentage and represented by following formulae [23].

$$\mathbf{MI} = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ observed} \text{ x } 100$$

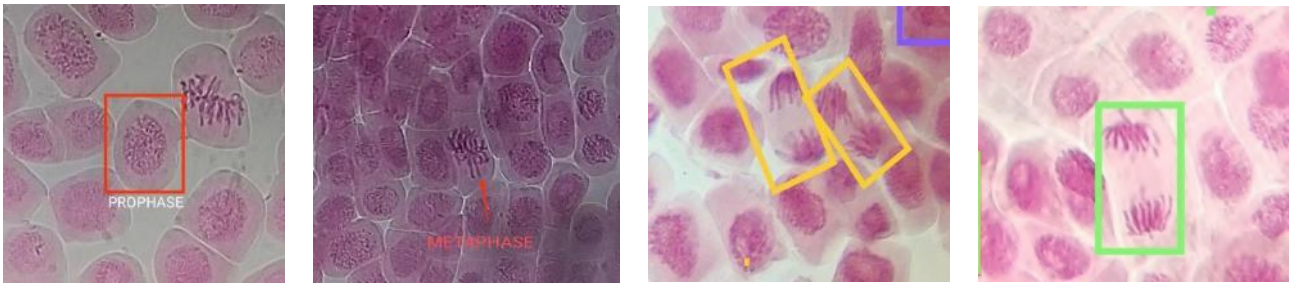
3. STATISTICAL ANALYSIS:

The data are expressed as Mean ± SE and statistical analysis was performed by using t-test.

Table1: Effect of Van Tulsi (5%) on mitotic index in onion root- tip cells at 72 hrs and 96 hrs.

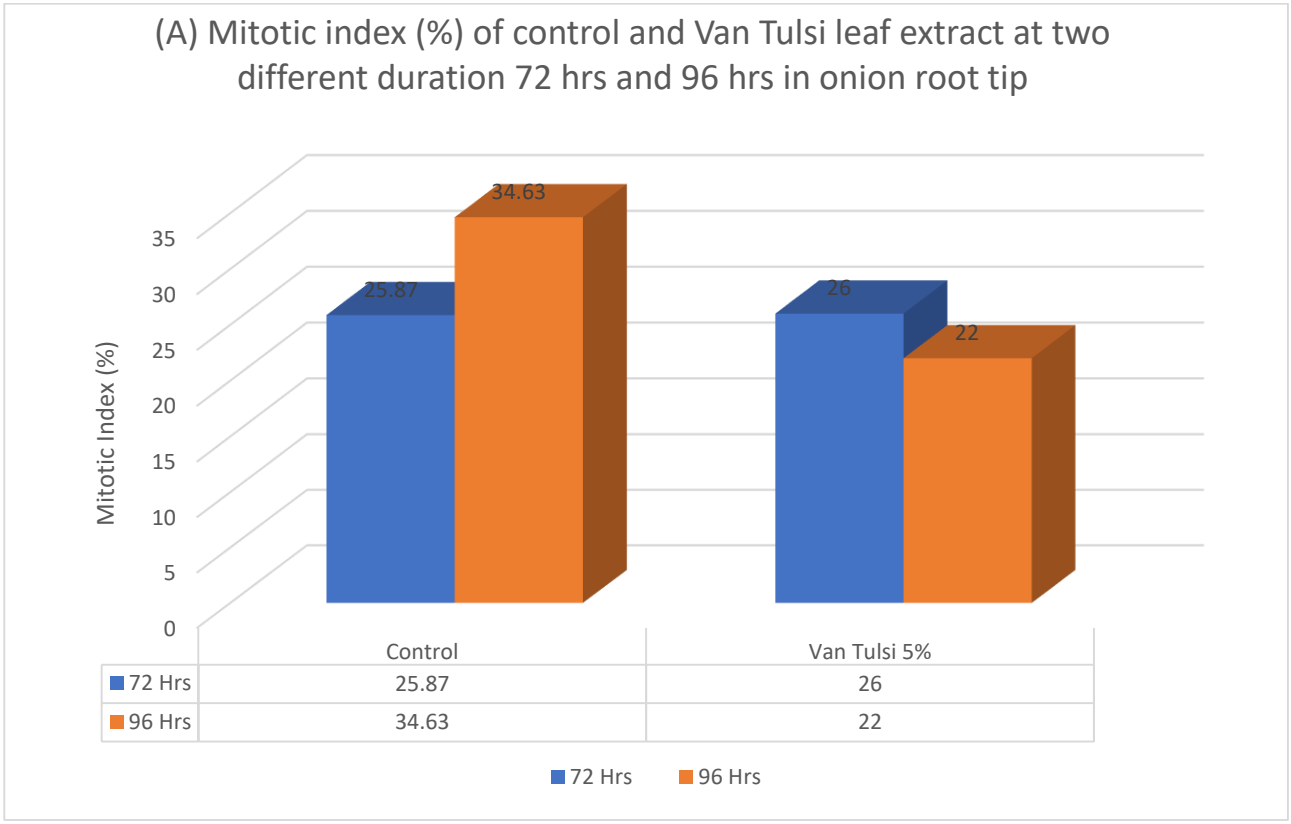
Exp Variant	Duration (hrs)	Total No of Cells Scored (N)	Total No of Dividing cells	Mitotic Index(%±S.E.)	Phase Distribution			
					Prophase (% ± S.E.)	Metaphase (% ± S.E.)	Anaphase (% ± S.E.)	Telophase (% ± S.E.)
Control	72	3305	855	25.87 ± 0.76	22.72 ± 0.73	1.75 ± 0.23	0.64 ± 0.14	0.76 ± 0.15

V (5%)	72	4853	1262	26.00 ± 0.63	23.10 ± 0.61	1.57 ± 0.18	0.74 ± 0.12	0.60 ± 0,11
Control	96	4014	1390	34.63 ± 0.75	29.42 ± 0.72	3.26 ± 0.28	0.97 ± 0.15	0.97 ± 0.15
V (5%)	96	4000	884	22.00 ± 0.65*	18.7 ± 0.62*	2.10 ± 0.23*	0.70 ± 0.13	0.63 ± 0.13
*- Indicate significant difference with control								

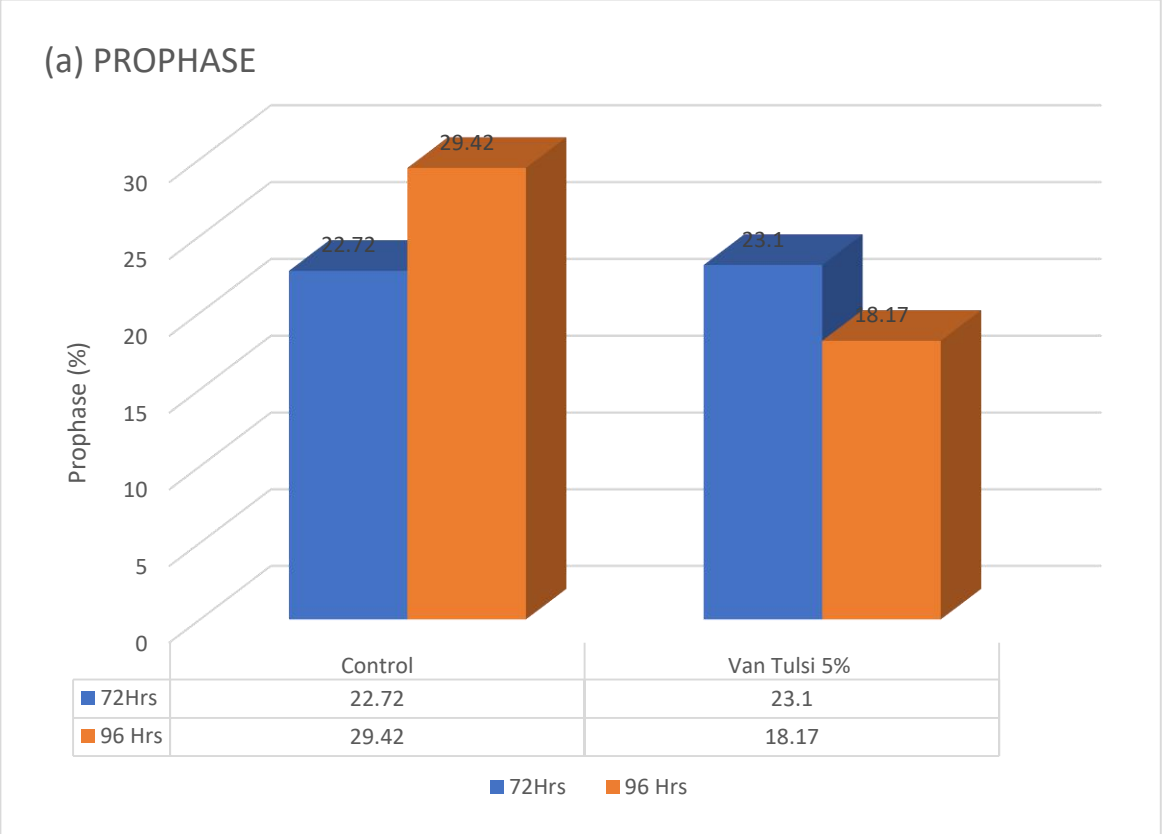


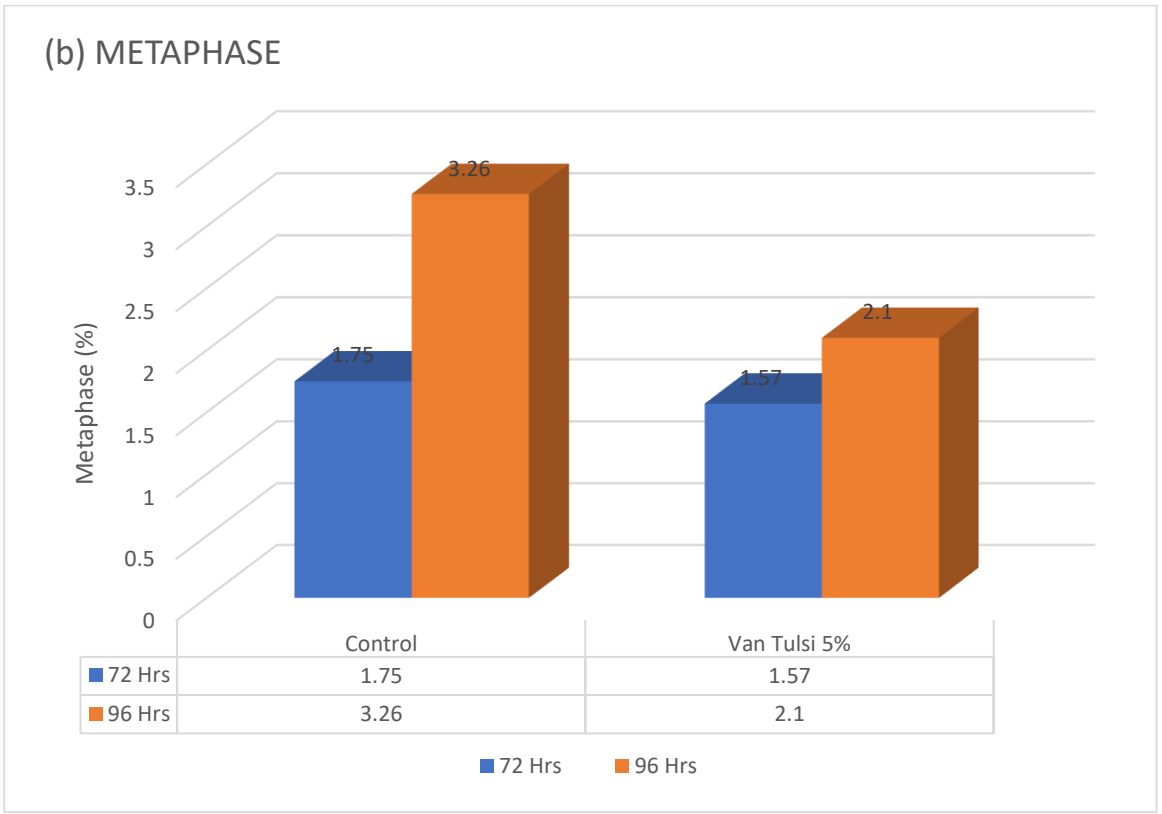
Prophase Metaphase Anaphase Telophase

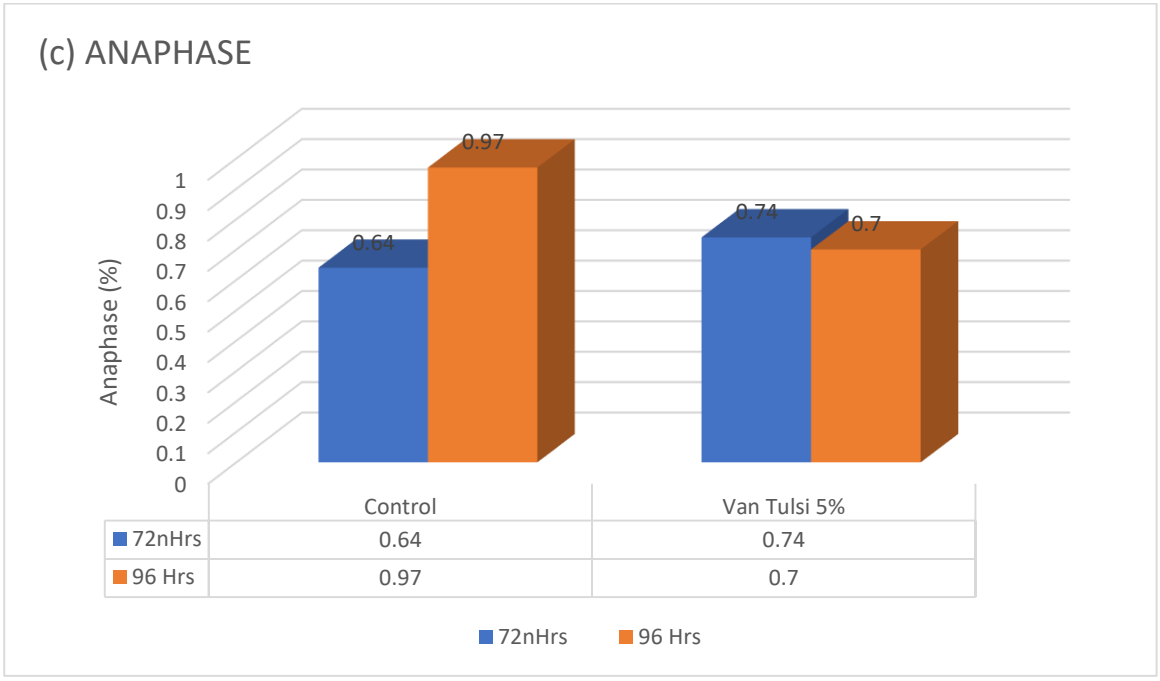
Figure 2: Different stages of Mitotic division in Onion Root Tip cells.



(B) PHASE DISTRIBUTION OF CONTROL AND VAN TULSI LEAF EXTRACT AT TWO DIFFERENT DURATION 72 HRS AND 96 HRS IN ONION ROOT TIP CELLS.


(a) PROPHASE
Prophase (%)
0
5
10
15
20
25
30
22.72
29.42
23.1
18.17
Control
Van Tulsi 5%
72Hrs
22.72
23.1
96 Hrs
29.42
18.17
72Hrs
96 Hrs


(b) METAPHASE
Metaphase (%)
0
0.5
1
1.5
2
2.5
3
3.5
1.75
3.26
1.57
2.1
Control
Van Tulsi 5%
72 Hrs
1.75
1.57
96 Hrs
3.26
2.1
72 Hrs
96 Hrs



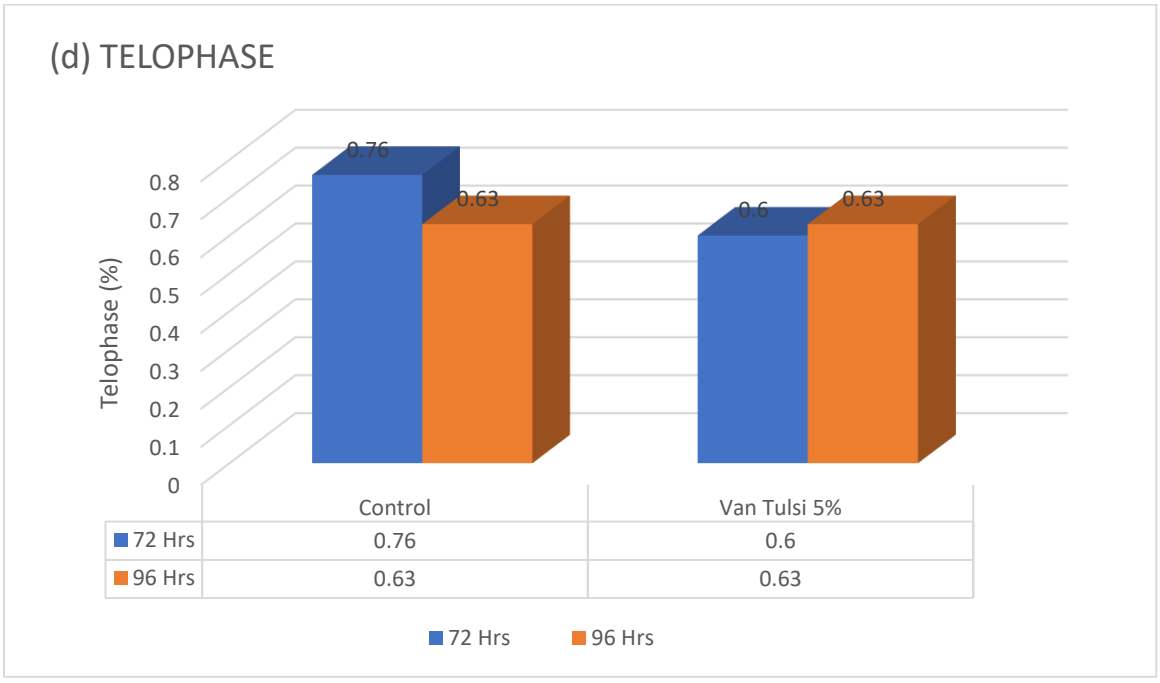


Figure 3: Histogram showing (a) Mitotic index (b) Phase distribution (Prophase; Metaphase; Anaphase; Telophase) in Onion Root Tip Cells at 72 hrs and 96 hrs in Control and Van Tulsi (5%) Leaf extract Treated group

4. RESULT AND DISCUSSION:

In 72 hrs treatment duration there is no significant difference were found in mitotic index and phase distribution of Van Tulsi treated onion root tips cells than control group of onion root tips cells. This suggest that 72 hrs concentration of Van Tulsi could not induce cytotoxicity.

In 96 hrs treatment duration, the mitotic index significantly decreased from 34.63% to 22.00% and in phase distribution mitotic index of Prophase and Metaphase significantly decreased from 29.42 to 18.7% and 3.26 to 2.10%. This decrease in mitotic index was mainly due to a decrease in the population of cells belonging to Prophase and Metaphase (Table 1).

Similar results were observed [23]. Numerous studies have shown that wherever there is root growth inhibition in Allium test, there is also reduction in the number of dividing cells [24].

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