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Marigold (Tegetes erecta) leaf extract as mitotic inhibitor on Onion (Allium cepa) root tip cells.

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Abstract: *The present work was made to evaluate the cytotoxic effects of Marigold leaf extract at two different duration (72 hrs and 96 hrs) on onion root tip cells. In 72 hrs treatment duration mitotic index significantly increased but at 96 hrs duration mitotic index significantly decreased due to decrease in the proportion of prophase cell population. Thus, leaf extract of marigold act as mitotic inhibitor on onion root tip cells at higher treatment duration.*

Keywords: *Mitotic index, Allium cepa, Cytotoxic, Tagetes erecta.*

1. INTRODUCTION:

Tagetes erecta, the medicinal herbs 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 Tagetes genus in organic agriculture is described in different works especially in the culture of vegetables due to its bactericidal, nematocidal, fungicide and insecticidal action [1], [2], [3], [4], [5]. Species of the Tagetes genus, especially the essential oil is attributed and is popular for having medicinal properties such as anti-biotic, anti-microbial, antispasmodic [8], immune stimulant, laxative, anthelmintic [13] antimycotic, analgesic [9],[10] and antioxidant [9] properties. It also exhibits anti-inflammatory, anti-depressant, anti-bacterial [11] properties.

It is used as a food colour in the African countries because of its richness in carotenoid leutin [12].

Tagetes erecta, commonly known as marigold, belongs to the family Asteraceae. TE is exploited to treat bronchitis, cold, rheumatic pain, headache, ulcers, respiratory diseases[6], and Diabetes mellitus[7],[23].

The leaves of Marigold are reported to be effective against piles, kidney troubles, muscular pain, ulcer and wounds. The pounded leaves are used as an external application to boils and carbuncles [22]. Scientific research has shown that many plants used in traditional and folk medicine are potentially toxic, mutagenic and carcinogenic [14],[15],[16].

Therefore, present study aims to evaluate the cytotoxic effect of leaf extract of *Tagetes erecta* on *Allium cepa*.

2. MATERIALS AND METHODS:

The cytotoxic effects of aqueous leaf extracts of *Tagetes erecta* was evaluated in *Allium cepa*. The leaf extract was prepared and higher concentration leaf extract were used.

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 10% Marigold leaf extract for 72 hrs and 96 hrs respectively and used as treated group.

SLIDE PREPARATION:

After treatment, slides were prepared by Acetocarmine squash preparation [17]. Approximately 3000 cells were randomly analysed 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 method was used 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 [18].

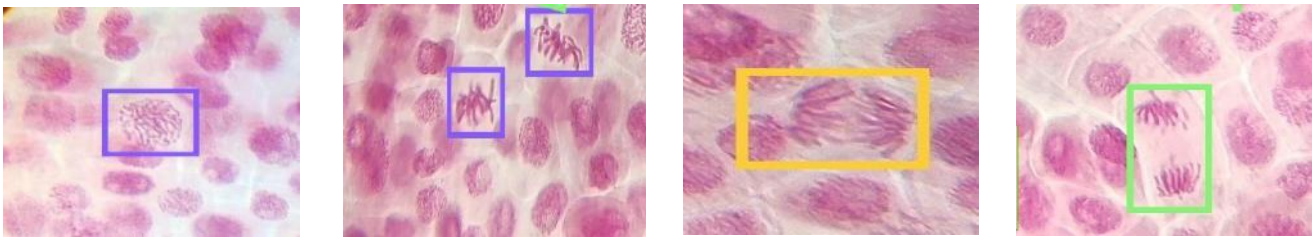
$$\mathbf{MI} = \frac{Total\ number\ of\ dividing\ cells}{Total\ number\ of\ cells\ observed} \text{ x } 100$$

3. STATISTICAL ANALYSIS:

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

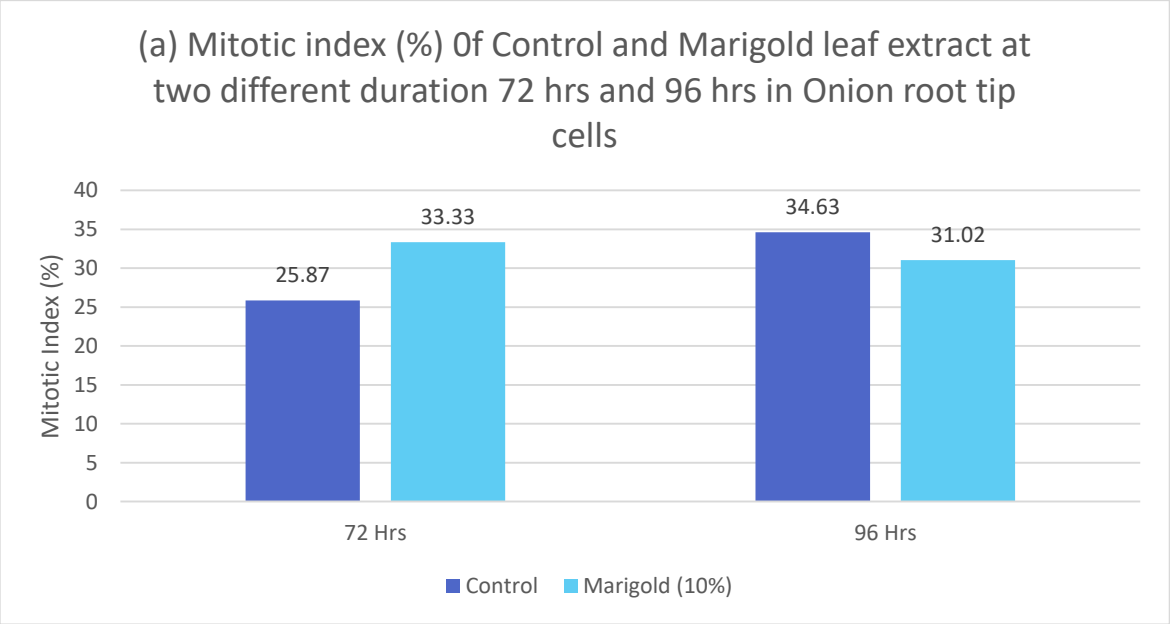
Table1: Effect of marigold (10%) on mitotic index in onion root- tip cells at 72 hrs and 96 hrs.

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
M (!0%)	72	2880	960	33.33± 0.88*	26.56 ± 0.82*	4.34 ± 0.38*	1.49± 0.23*	0.94 ± 0,18
Control	96	4014	1390	34.63 ± 0.75	29.42 ± 0.72	3.26 ± 0.28	0.97 ± 0.16	0.97 ± 0.15
M (!0%)	96	2740	680	31.02± 0.88*	24.82± 0.83*	3.87 ± 0.37	1.24 ± 0.21	1.09± 0.20
*- Indicate significant difference with control								

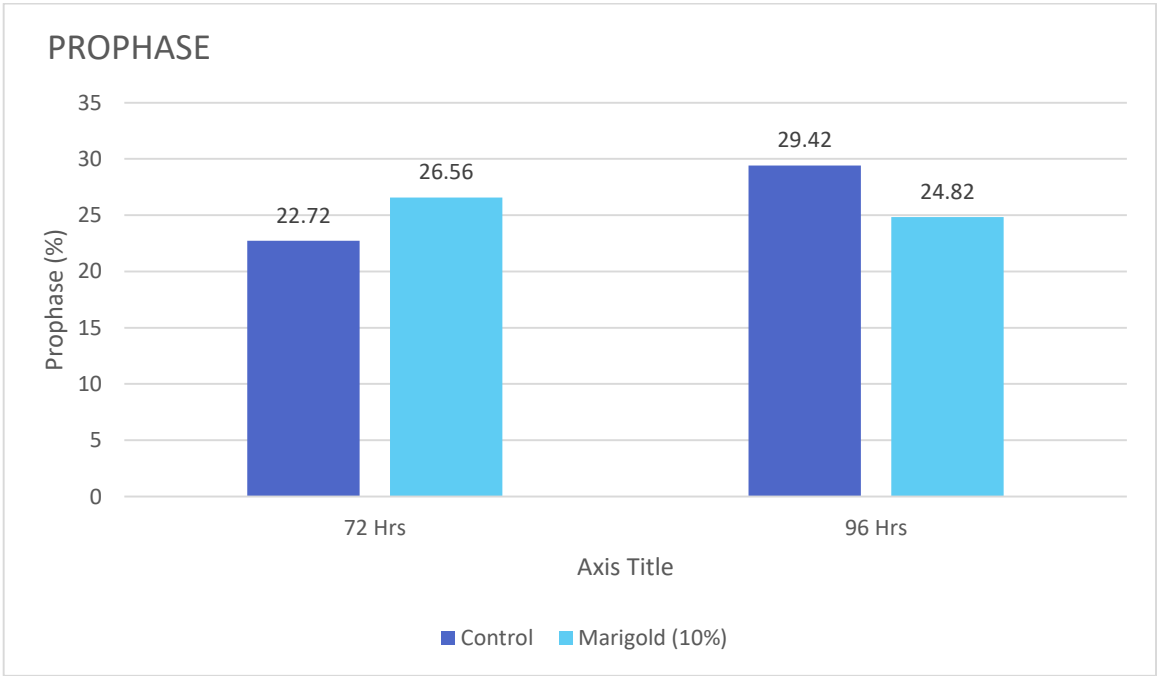


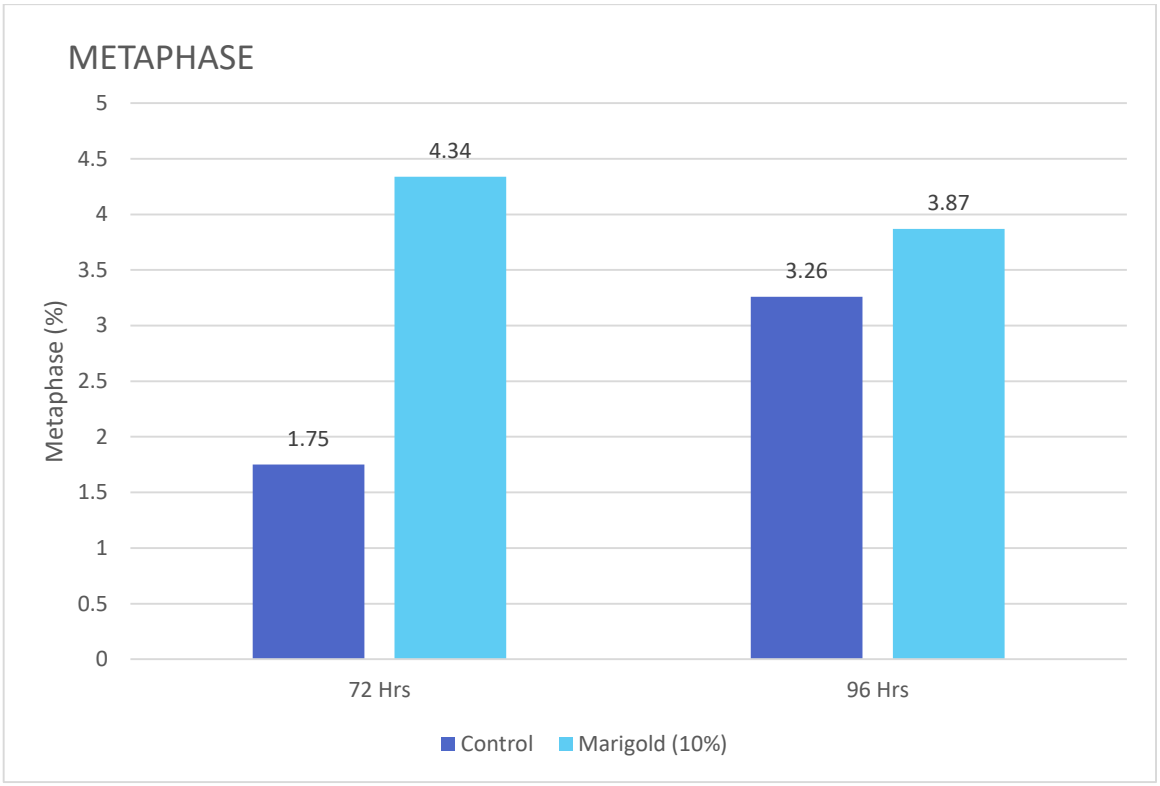
Prophase Metaphase Anaphase Telophase

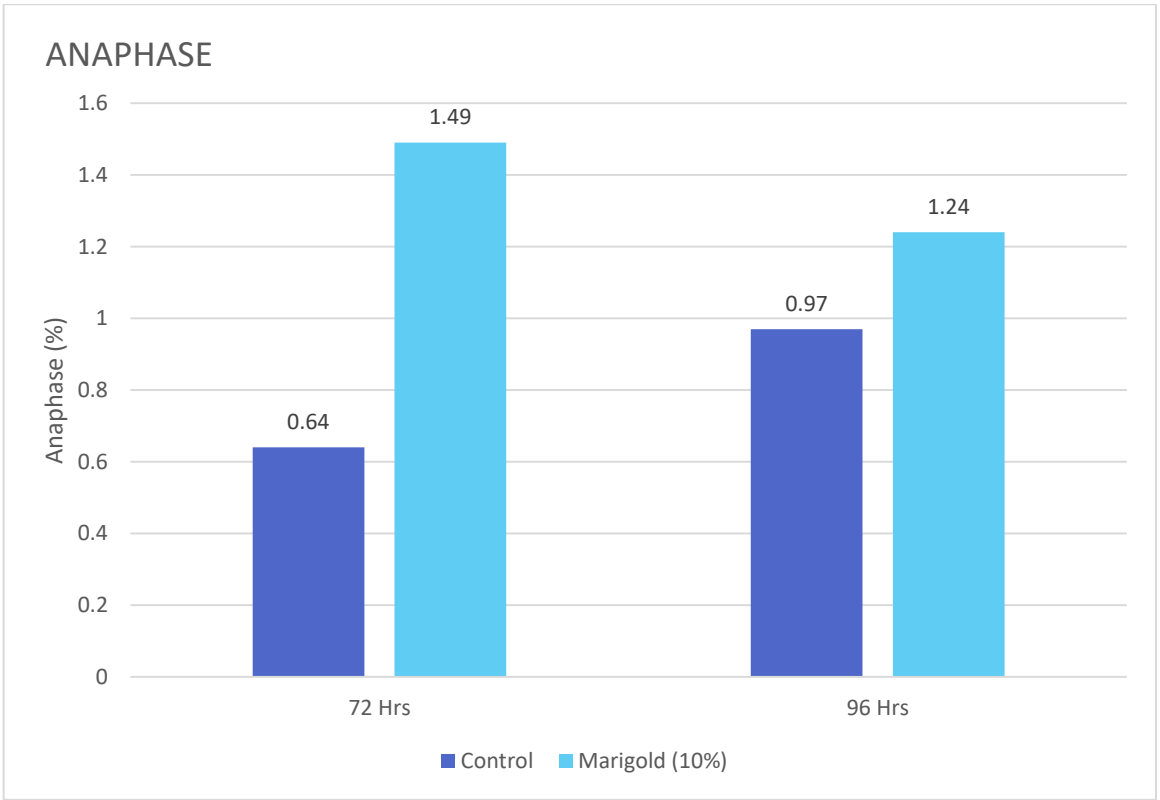
Different stages of Mitotic division in Onion Root Tip cells.

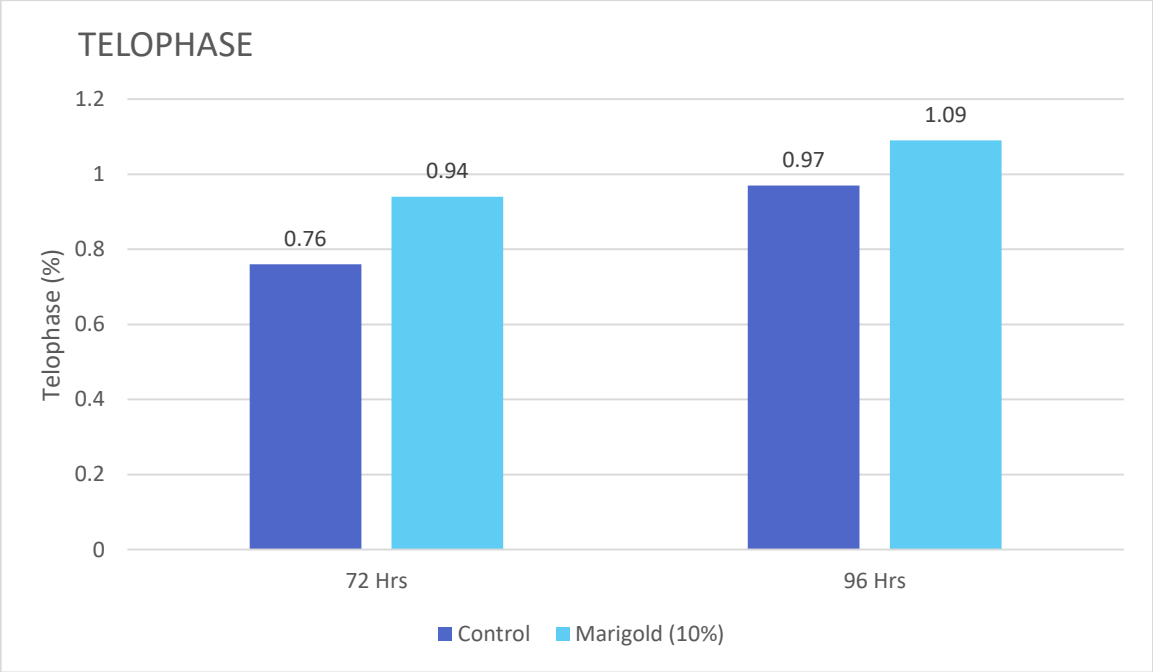


(b) PHASE DISTRIBUTION OF CONTROL AND MARIGOLD LEAF EXTRACT AT TWO DIFFERENT DURATION 72 HRS AND 96 HRS IN ONION ROOT TIP CELLS.




METAPHASE
Metaphase (%)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
4.34
3.87
3.26
1.75
72 Hrs
96 Hrs
Control
Marigold (10%)


ANAPHASE
Anaphase (%)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1.49
1.24
0.97
0.64
72 Hrs
96 Hrs
Control
Marigold (10%)



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 Marigold (10%) Leaf extract Treated group

4. RESULT AND DISCUSSION:

In 72 hrs treatment duration mitotic index significantly increased from 25.87 % to 33.33%. In phase distribution, the percentage of mitotic index of prophase is also increased from 22.72% to 26.56%. The increase in mitotic index at 72 hrs treatment of Marigold due to increase in population of cell belonging to Prophase. This suggest that 72 hrs concentration of Marigold could not induce cytotoxicity.

In 96 hrs treatment duration, the mitotic index significantly decreased from 34.63% to 31.02% and in phase distribution mitotic index of Prophase decreased from 29.42% to 24.82%. This decrease in mitotic index was mainly due to a decrease in the population of cells belonging to Prophase (Table 1).

MI measures the proportion of cells in the M-phase of the cell cycle and its inhibition could be considered as cellular death or a delay in the cell proliferation kinetics [19].

Numerous studies have shown that whenever there is root growth inhibition in the Allium test, there is also reduction in the number of dividing cells [20]. Thus, the Marigold leaf extract (10%) showed cytotoxic effect at 96 hrs.

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