



Cytotoxic effect of marigold (*Tagetes erecta*) leaf extract on root-tip cells of onion (*Allium cepa*).

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Abstract: The present work is designed to evaluate cytotoxic effects of leaf extracts of *Tagetes erecta* at 5% concentration during 72 hrs and 96 hrs on *Allium cepa* root tip cells. When the bulbs of *Allium cepa* treated with leaf extract of *Tagetes erecta* (5%) for 72 hrs an increase in mitotic index were observed due to increase in the proportion of Prophase cell population. But when bulbs were treated *Tagetes erecta* (5%) for 96 hrs a decrease in mitotic index were observed due to decrease in the proportion of Prophase and Metaphase cell population. The results showed that *Tagetes erecta* (5%) act as mitotic inhibitor at 96 hrs duration.

Key Words: Mitotic index, *Allium cepa*, Cytotoxicity, *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].

Tagetes erecta, commonly known as marigold, belongs to the family Asteraceae. TE is exploited to treat bronchitis, cold, rheumatic pain, headache, ulcers, and respiratory diseases[6],[7]. Essential oil of *Tagetes erecta* can be attributed to its properties like anti-biotic, anti-microbial, anti-parasitic, anti-septic, anti-spasmodic [8] and antioxidant [9].

The leaves 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. It is reported to have antioxidant, antimycotic, analgesic activity [9],[10]. It also exhibits anti-inflammatory, anti-diabetic, anti-depressant, anti-bacterial and insecticidal, activity [7],[11].

Tagetes erecta is used as a food color in the African countries because of its richness in carotenoid leutin [12]. Species of the *Tagetes* genus is popular for having medicinal properties, such as analgesic, antispasmodic, immune stimulant, laxative and anti-helminthic [13] and bactericidal [3],[4]. 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 [17] and 5% leaf extract were used [18].

The onion bulb weighing approximately 20-30 grams were purchased from local market and their roots were initially allowed to grow till 1 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% Marigold leaf extract for 72 hrs and 96 hrs respectively and used as treated group.

2.1 SLIDE PREPARATION:

After treatment, slides were prepared by Acetocarmine squash preparation [19]. Approximately 4000 cells were randomly analysed in both control and treated group of onion bulbs.

Frequency of Mitotic index and Phases distribution were calculated.



2.2 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 [20].

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

3. STATISTICAL ANALYSIS:

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

Table1: Effect of marigold (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(% \pm S.E.)	Phase Distribution			
					Prophase (% \pm S.E.)	Metaphase (% \pm S.E.)	Anaphase (% \pm S.E.)	Telophase (% \pm S.E.)
Control	72	3305	855	25.87 \pm 0.76	22.72 \pm 0.73	1.75 \pm 0.23	0.64 \pm 0.14	0.76 \pm 0.15
M (5%)	72	3440	1006	29.24 \pm 0.78*	25.52 \pm 0.74*	1.95 \pm 0.24	0.87 \pm 0.16	0.90 \pm 0.16
Control	96	4014	1390	34.63 \pm 0.75	29.42 \pm 0.72	3.26 \pm 0.28	0.97 \pm 0.16	0.97 \pm 0.15
M (5%)	96	4310	854	19.81 \pm 0.61*	15.75 \pm 0.55*	2.51 \pm 0.24*	0.95 \pm 0.15	0.60 \pm 0.12

* Indicate significant difference with control.

4. RESULTS AND DISCUSSION:

In 72 hrs treatment duration mitotic index significantly increased from 25.87 % to 29.24%. In phase distribution, the percentage of mitotic index of prophase is also increased from 22.72 to 25.52%. 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 19.81% and in phase distribution mitotic index of Prophase and Metaphase also decreased from 29.42 to 15.75 and 3.26 to 2.51 respectively. This decrease in mitotic index was mainly due to a decrease in the population of cells belonging to Prophase and metaphase (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 [21]. An aqueous extract of Marigold may possess ability to block the synthesis of DNA, a phenomenon that has been reported with extract of other medicinal plant [22]. 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 [23]. Thus, the Marigold leaf extract (5%) showed cytotoxic effect at 96 hrs only.

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