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Evaluating the toxic effects of *Ficus infectoria* Roxb. and *Emblica officinalis* Gaertn. leaf extracts on cell division and chromosomal morphology of *Cicer arietinum* L.

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Keywords

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Abstract

In the present study, the aqueous extracts of *Ficus infectoria* and *Emblica officinalis* leaves were evaluated for their toxic effect on cell division and chromosomal morphology of *Cicer arietinum* root apical meristem. The extracts were prepared by dissolving 15 gm, 30 gm and 45 gm of dry leaf powder in 1000 ml of double distilled water. The experiment was conducted in sterilized petri dishes. The results revealed that the different concentrations of aqueous extract of *F. infectoria* and *E. officinalis* caused cytotoxic and mitodepressive effects on chromosome of *Cicer arietinum.* The dose–dependent and statistically significant ($p<0.05$) inhibitation of mitotic index by the extracts were noticed when compared with the control. These results indicate the inhibitory, mitodepressive and turbagenic activities of the aqueous extracts of studied plants on *Cicer arietinum.*

1. Introduction

Recent development in forest management has expanded its scope to accommodate human agricultural activities. Consequently, agroforestry systems based on tree planting have been developed in order to improve their economic and ecological role. However, the harmful effects of forest trees on agricultural crops growing around them adversely affected through release of allelochemicals into the crop environment. These allelochemicals (mostly secondary metabolites) affect the growth of other crops growing near to it. Some of the important tree species distributed in semi– arid region of India include *Ficus infectoria* and *Emblica officinalis*. These species are incorporated in agroforestry programs as an associated species, but it seems that they have some inhibitory effect on agricultural crops and on ground vegetation which might have been caused either by fallen leaves or plant leachates or root exudates. These fallen leaves can be detrimental to other plants and play an appreciable role in the distribution of vegetation and the yield of various crops [1-4]. Many workers have reported this inhibitory effect of allelopathy in agricultural and forestry [5-7, 26-30]. Some species currently used in agro forestry systems reportedly have allelopathic properties. [8-9].

Hence it seems essentials that the allelopathic compatibility of crops with trees should be checked before being introduced to agroforestry systems. Keeping these views in mind, the present study was undertaken in order to elucidate the allelopathic potentials of different concentration of *Ficus infectoria* and *Embilica officinalis* leaf extracts on the largest grown leguminous crop of India i.e. *Cicer arietinum* .

2. Materials and Methods

2.1. Procurement of Seeds:

Seeds of *Cicer arietinum* were obtained from Agriculture seed store, Jhansi (U.P.), India and lab-scale study was carried out in 2009 at Department of Botany, Bundelkhand University, Jhansi (India).

2.2. Aqueous Leaf Extracts Treatments:

Healthy uniform size seeds were selected and presoaked in distilled water for 3 h and then socked in 15gL-1, 30gL^{-1} and 45gL^{-1} of different aqueous extracts for 3 h. Control group were soaked in distilled water for 3 h.

2.3. Cytogenic Analysis:

The Cytogenic analysis was carried out in root tips of germinated seeds, treated with different concentrations of aqueous extracts of *F. infectoria*, and *E. officinalis*. Chromosome preparations were made from the root tips using the method described by Qian [10] with minor modifications [11]. The root tips were cut and fixed in Carnoy's fixative (Anhydrous alcohol: Glacial acetic acid, 3:1) for 24 h, transferred to 70 % alcohol and stored in a refrigerator until use. Root tips were hydrolyzed in 1N HCL for 15 min at root temperature and then stained with acetocarmine for 30 min. Chromosome spreads were made which using the squash technique [12]. To overcome observer's biasness, all slides were coded and examined blind. Total 100 cells were scored from each preparation to study the mitotic index (MI) and various chromosomal aberrations such as fragments, precocious separations, sticky chromosomes, laggards, bridges were studied 50-cells in meta – anaphase plates.

2.4. Statistical analysis

One way ANOVA test was performed using GPIS software 1.13 (Graphpad, California, USA) in order to detect the significance of differences of variables. All values are expressed in Table as mean ±SE.

3. Results

MI which indicates the cell division frequency was determined for untreated as well as the seeds treated with aqueous leaf extracts of different tree species:

Table 1 Effect of aqueous leaf extract of *ficus infectoria* on chromosome aberrations of *Cicer arietinum*

Treatment	Abnormal Cell/50	Micro-nucleus	Stickiness	Lagging	C-Mitosis	Precocious separation	Fragment	Bridge	Vagrant	Chromosomal aberration
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	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	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
15 g/L	7	0.8 ±0.16	0.40 ± 0.37	0.0 ± 0.0	0.40 ± 0.01	0.20 ± 0.07	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.8 ±0.61
30 g/L	15	0.92± 0.58	1.0 ± 0.67^{c}	0.0 ± 0.0	0.20 ± 0.01^{b}	0.60 ± 0.24^{bc}	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.72 ± 1.50^{b}
45 g/L	20	1.6 ± 0.01	1.20 ± 0.09^{ab}	1.20 ± 0.37	1.0 ± 0.48^{bc}	1.40 ± 0.27^{abc}	0.0 ± 0.0	0.40 ± 0.24	0.0 ± 0.0	6.80 ± 1.46^{bc}

$P^{a} < 0.001$; $P^{b} < 0.01$; $P^{c} < 0.05$ compare to control.

Table 2 Effect of aqueous leaf extract of *Emblica officinalis* on chromosome aberrations of *Cicer arietinum*

Treatment	Abnormal Cell/50	Micro-nucleus	Stickiness	Lagging	C-Mitosis	Precocious separation	Fragment	Bridge	Vagrant	Chromosomal aberration
		Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	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	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
15 g/L	12	0.0 ± 0.0	0.60 ± 0.24	0.0 ± 0.0	1.0 ± 0.01	0.20 ± 0.40	0.60 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	2.4 ± 0.7
30 g/L	28	0.20 ± 0.01	1.20 ± 0.37^{ab}	0.80± 0.48	1.8 ± 0.31^{bc}	1.2 ± 0.20^{abc}	0.40 ± 0.09	0.40 ± 0.14	0.0 ± 0.0	5.64 ± 1.6
45 g/L	39	0.60 ± 0.24	1.80 ± 0.37^{bc}	0.83 ± 0.37^{b}	1.20 ± 0.24	0.80 ± 0.24	1.2 ± 0.34	0.80 ± 0.20	0.80 ± 0.37^{bc}	8.30 ± 3.17^{bc}

$P^{a} < 0.001$; $P^{b} < 0.01$; $P^{c} < 0.05$ compare to control.

Effects of aqueous extracts on Mitotic Index of *Cicer arietinum*:

Ficus infectoria

The Mitotic Index (expressed in percentage) is depicted in Fig-1. Control group revealed mitotic index of 15.33 ± 1.15. There is a dose dependent decline in the MI of all the treated groups. At the lowest concentration (15gL^{-1}) the MI was 6.6 ± 0.57, which further decreased to 6.33 ± 0.57 at 30 g L^{-1} and at higher concentration (45gL^{-1}) the lowest MI (4.66 ± 1.15) was observed. All three concentrations showed highly significant (P<0.001) reduction in MI when compared to control group.

Emblica officinalis

Treatment of *Cicer arietinum* with various concentrations *E. officinalis* resulted in a dose - dependent decrease in the MI at all the treated groups. The untreated groups yielded a MI value of 17.33 ± 1.15. At lower concentration, reduction in MI value (8.66 ± 0.57) was non significant, however at 30gL^{-1}, MI (7.33 ± 1.32) showed highly significant (P<0.001) reduction when compared to control. At higher concentration (45gL^{-1}) the MI was (3.33 ± 0.57) which is highly significant when compared to control group. Fig .1.

Fig.1. Mitotic index (%) in root tips of *Cicer arietinum* treated with aqueous extract of *Ficus infectoria.* Different letter indicate significant differences. Bar indicates S.D.

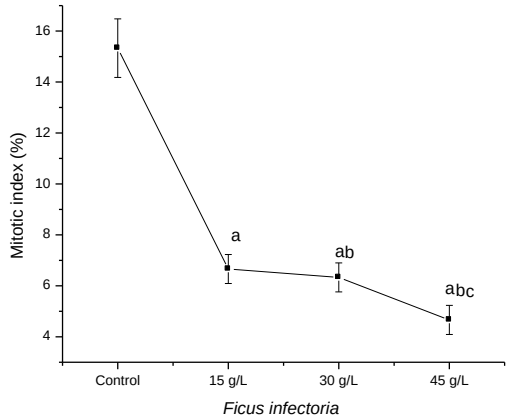
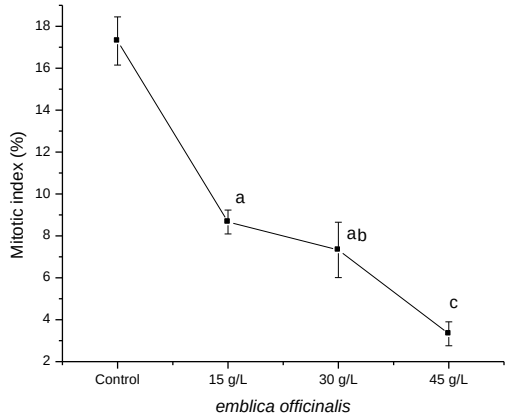


Fig 2. Mitotic index (%) in root tips of *Cicer arietinum* C-235 treated with aqueous extract of *Emblica officinalis.* Different letter indicate significant differences. Bar indicates S.D



Chromosomal aberrations

Ficus infectoria:

Teatment of *Cicer arietinum* with various concentrations of aqueous leaf extract of *Ficus infectoria* resulted in dose-dependent increase in the frequency of chromosomal aberrations. The frequency of total number of aberrations was very significantly ($P<0.01$) higher after treatment of 30g L^{-1} aqueous extracts. The highest concentration 45 gL^{-1} posed an increase of 6 folds. The major aberration observed in excess of spontaneous occurrence was micronuclei, lagging chromosomes were present only at higher concentration (45gL-1). The prevalence of abnormalities was in the following order:

Micronuclei > Precocious Separation > Stickiness > Lagging chromosome > C-mitosis > Bridge

Embilica officinalis:

Aqueous extract exhibited a significant increase in the percentage of aberrant metaphase-anaphase plate. Aqueous extract produced a dose – dependent increase in the percent of aberrant cells. Stickiness- as the major type of aberration but difference was significant from normal only at higher concentrations (30gL^{-1} and 45gL^{-1}). Vagrant chromosome was observed only at higher concentration.

The frequency of abnormalities was in the following order:

Stickiness >Fragment >C-mitosis

Precocious separation= Lagging chromosomes = Bridges > Micronuclei.

4. Discussion

The aqueous leaf extract of two species *F. infectoria and E. officinalis* produces a hindrance in cell division and increases clastogenic effects such as sticky chromosome, bridges and fragments. These extracts caused decrease in mitotic index due to increased number of interphase or dead cells and accumulation of interphase cells may be due to inhibitation of DNA synthesis [13]. The effect of aqueous extract might interact with DNA subsequent mitotic inhabitation, as reported in case of food preservatives [14].

Other investigation indicated that mitotic inhibition could be due to change in duration of different stages of all cycle such as G_2 period [15-16] or 'S' phase duration [17-18]. The clastogenic effects caused by the extracts from tree species include bridges, fragments, stickiness, lagging chromosome, vagrant, precocious separation, micronuclei and C-mitosis. Sticky chromosomes have sticky "surface" which causes chromosome organization. Chromosome fragments indicate chromosome breaks, and results in formation of bridges (19). Adhesion of the centromers of one or more chromosomes to the outer layer of the plasma and movement of the others towards the equatorial plate led to appearance of lagging chromosome (20-23).

Cells containing non-congression metaphase where the whole chromosome has tendency to build up the equatorial plant, a part from one or more chromosomes lie free in the cytoplasm, which result in formation of micronuclei. It was also used as a reliable parameter in *Vicia faba* test system (24).

Thus, aqueous leaf extracts of tree species appeared to hinder cell division, increasing the number of interphase cells along with inducing clastogenic effects. Therefore it is concluded that undertaken tree species are potential sources of biologically active substance with possible applications in biology medicine and agronomy.

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