

## MITOTOXIC EFFECT OF PONGAMIA PINNATA ON VICIA FABA SEEDS

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### ABSTRACT

The present experiment is undertaken to determine the allelotoxic effect of pongamia extract on cytomorphology of *Vicia faba* L. The seeds were treated with different concentrations (10, 25, 50 and 75 %) of Pongamia extract for 10h. A significant reduction in mitotic index was observed in seeds exposed to Pongamia extract compared to control which decreased with an increase in concentration of Pongamia extract. On the basis of these results, it was concluded that all pongamia extract concentrations significantly affect the cytomorphology of *V. faba*, while higher concentrations of pongamia extract were found to be more mutagenic and cytotoxic. In present study the cytotoxic effects of the crude extracts of Pongamia on the *Vicia faba* L. seeds for 10h were evaluated. In the first experiment, the Pongamia caused losing in the mitotic index accompanied with considerable percentage of chromosomal aberrations. These abnormalities include stickiness, disturbed chromosomes, bridges, lagging chromosomes and micronuclei. The mitotic index decreases due exposure plant extract in higher concentration that is 12.00 at 75% concentration. The minimum value of mitotic inhibition frequency in *Vicia faba* root tips was 6.08% and scored at 10 % concentration for whereas the maximum value of mitotic inhibition frequency was 32.33% at 75% concentration for 10 hrs treatment. Pongamia showed mild reducing effect on chromosomal aberrations at 800ppm concentrations for 8hrs which were 1.95% abnormal metaphase, 2.09% abnormal anaphase, 3.98% stickiness, 5.97% fragmentation, 2.96% late separation, 2.89% precocious movement, 5.99% abnormal c-mitosis, 2.84% bridges, 14.01% laggards

**Keywords:** Pongamia , Mitotoxic effect.

### INTRODUCTION

The *Pongamia pinnata* seeds contain about 40% oil, which can be converted to biodiesel by trans esterification method (Meher *et al* 2006). The seeds of *Pongamia pinnata* contain 30 to 40% oil) .which can be converted to biodiesel by transesterification with methanol in the presence of Potassium hydroxide (Sarma *et al* 2005), Ahmad *et al* 2003). Recent investigations have revealed that many

plants used as food or in traditional medicine have mutagenic effects and cytotoxic and genotoxic effects in vitro and in vivo assays .Many plants contain mutagenic and/or carcinogenic substances and their use has been correlated with high rate of tumor formation in some human populations (M. T. Brito 1990) . *Vicia faba* L. (2n = 12), of the family Fabaceae, have homozygous genotype because of self pollination (Gulfishan *et al*, 2010). Thus, *Vicia faba* based bioassays have established role to study

chromosome anomalies due to their large and visible chromosome (Asthana and Kumar 2014).

## MATERIALS AND METHODS

The leaves were dried and pulverized, using mortar and pestle in the laboratory following the method of Mukhtar and Tukur (1999). Thereafter, extracts of the extracts of leaves were prepared. The extracts were serially diluted to the concentrations of 0, 25, 50 and 75 % respectively For mitotic analysis the Root tips of healthy seeds of *Vicia faba* were germinated on moist filter paper in petridishes . Then germinated seeds having root tips of about 0.5 to 1 cm length were cut from seedlings and transferred into varying concentrations (5% to 50%) of extracts for 10 hrs duration (Haroun and Al Shehri 2001). Excised tips were fixed in freshly prepared fixative solution acetic alcohol (1:3) for 24hrs. Fixation and Staining of Root Tips: For preparation of metaphase chromosomes, root tips were cut and fixed overnight in Carnoy's fixative and were kept at 4° C until later use. For preparing smears the root tips were transferred to room temperature in distilled water for 5 min. The root tips were then hydrolyzed in 1 N HCl at 60°C for 7 min. Preparation of slides from the fixed root tips was done following acetocarmine squash technique (1% solution of carmine in 45% acetic acid). Freshly fixed materials were transferred into 1% acetocarmine for at least 30 min and then were analyzed by the squash method. The untreated and treated seeds of *Vicia faba* varieties were sown in the field/ experimental plots as well as in the pots to study the growth

performance at the outset. The experimental materials used in the present study were grown in the experimental plots in the Randomised Block Design with three replications in the Rabi season. The seed treatments were done in order to assess the suitability and impact on the growth and morphological behavior. Samples were collected and observations on *Vicia faba* varieties were made.

## RESULT AND DISCUSSION

The results revealed that in *Vicia faba* treated root tip cells, the metaphase stage was also the most affected stage by extract treatment and the total percentage of its abnormalities was higher than those at other mitotic stages. In this respect, the total percentage of abnormalities in the other mitotic stages was in the following sequence Prophase> anaphase>telophase for all treatments. Finally, this work provides the evidence for plant extract cytotoxicity in plant cells and also confirms the efficiency of the plant cytogenetic assays on monitoring the cytotoxicity of the plant extract. The mitotic index decreases due exposure plant extract in higher concentration that is 12.00 at 75% concentration. the minimum value of mitotic inhibition frequency in *Vicia faba* root tips was 6.08% and scored at 10 % concentration for whereas the maximum value of mitotic inhibition frequency was 32.33% at 75% concentration for 10 hrs treatment(Table:1). The reducing effect of mitotic index after treatment in all four concentrations 22 ,21.89, 21.54, 21.67 at 8 hrs exposure it as observed mitostatic effect was very low.

Treatment Conc. ( %)for Time (10hr.)	Mitotic phase % Prophase	Mitotic phase % Metaphase	Mitotic phase % Anaphase	Mitotic phase % Telophase	mitotic index (MI $\pm$ S.E)	% of Mitotic Inhibition
control	36.72	30.58	15.11	11.16	18.20 $\pm$ 0.62	0.00
10	37.94	52.87	3.42	1.22	17.69 $\pm$ 0.32	6.08
25	46.30	44.95	1.70	1.05	16.99 $\pm$ 0.15	10.61
50	46.71	40.11	7.77	0.00	14.50 $\pm$ 0.68	21.33
75	42.86	44.29	12.86	0.00	12.00 $\pm$ 0.36	32.33

**Table 1:** Frequency of mitotic phases and the percentage of total abnormal mitotic phases after treatment of *Vicia faba* root tips with different concentration of *Pongamia pinnata* extracts for 10 hours.

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