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MITOTIC EFFECTS AND GENOTOXICITY EFFECTS OF LEAF AND FLOWER EXTRACTS OF *AGERATUM CONYZOIDES* ON *ALLIUM CEPA*.

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Abstract:

The Ageratum genus belongs to the Asteraceae family. It is a medicinal herb that has traditionally to be used to treat dysentery and diarrhea in many traditional cultures. It's also a nematicide and a pesticide. The aim of this study was to investigate genotoxic effects of aqueous extracts (10,20,30,40 & 50) from *Ageratum conyzoides* leaf and flower extracts on *Allium cepa* root tip. For this purpose, *Allium cepa* onion bulbs were treated with the *Ageratum conyzoides* leaf and flower extracts for 24 h. The root tips from each bulb were excised and fixed in Carney's fixative for 24 hours. Mitotic preparation was made by hydrolysing the root tips in a mixture of 1 N HCL and 2 % acetocarmine (1:9), at 60±2°C for 2 hrs. The result of this study showed that aqueous extracts reduced mitotic index, but induced chromosomal aberrations and mitotic aberrations in comparison with control. Aqueous extracts induced disturbed metaphase, precocious movement, unorientation, laggard, stickiness, fragments, bridges, late separation, etc. furthermore, these effects were related to extract concentrations. These results showed that *Ageratum conyzoides* aqueous extracts have mitotic and genotoxic effects.

Key words: *Vicia faba* L., plant extract, genotoxic effect, *Allium cepa* L.

Introduction

Ageratum conyzoides is a tropical and subtropical herb that can be found in a variety of habitats. *Ageratum conyzoides* is a tropical American native that has become widely naturalized around the world. In India, it can be spotted in the Himalayas at elevations of up to 2,000 metres. It's a tropical annual herbaceous weed that grows in abundance. It is an erect, softly hairy annual plant that grows up to 2.5 feet tall. In immature plants,

the stem is aerial, cylindrical, green, and weak, but as the plant matures, it becomes somewhat brown and sturdy **[13]**. Leaves are oblong to lance-shaped, coarsely rounded, and serrated on opposite sides. A large number of pale blue or whitish flower heads, measuring 6 mm across, develop in domed to flat-topped clusters in leaf axils or at the ends of branches. Flowers are available for the majority of the year. The leaves are also covered with fine hair and have a mild fragrant disagreeable odour. The dark seeds are scaled and have needle-like ends. Ageratum is used in alternative medicine to treat epilepsy and wounds, as well as as an insect repellent. It's used to cure anything from common colds to headaches, boils, eczema, bleeding, wounds, and burns in China. This herb is used as a folk medicine in some countries to treat conditions like purgative, febrifuge, ophthalmic, colic, ulcers, and wounds **[11]**. **[8]** It's also used to protect plants and insects against disease. The Ageratum genus is a fast-spreading plant that is currently posing a significant concern for environmentalists, ecologists, farmers, and animal scientists. A number of research have been conducted on its weed control **[1] [9]**.

Allium cepa has proven to be an excellent material for studying the genotoxic effects of environmental mutagens in the short term. The Allium test is a standardised test for monitoring cyto-genotoxicity **[3]**, seems to have some advantages. One of the tests it performs is for genotoxicity. It's also simple to use, inexpensive, and has a strong connection with mammalian test methods. **[3],[5],[6],[2],[7].[15].** The results of the Allium test were also compatible with a test battery that included both prokaryotes and eukaryotes **[4]**.

In this study, we investigated genotoxic effects of aqueous leaf and flower extracts of *Ageratum conyzoides* on *Allium cepa* root tip using the Allium test.

Material and methods

Plant collection and extraction

In June-July 2019, *Ageratum conyzoides* was collected in the East Singhbhum area. After that, I collected 250 g of *Ageratum conyzoides* leaves and dried them separately at 60°C. After that, I ground it to pass through a 1 mm screen and stored it at room temperature. I made an aqueous plant extract with double distilled water in a 50:1 (V: W) water plant sample ratio, then chilled it for 18 hours. The suspension was then vacuum filtered through 0.4mm polycarbonate filters after being centrifuged at 1000gm for 15 minutes. The solutions of various concentrations were made from the mother solution by adding the appropriate amount of distilled water. The seed treatment lasted 12 hours.

Allium test

We used the Allium test to assess the genotoxicity of *Ageratum conyzoides* aqueous extracts **[4]**. Healthy and medium-sized bulbs of common onion (*Allium cepa*) were chosen for this purpose. Forceps were used to remove the loose outer scales of bulbs and old roots, exposing root primordia. A series of bulbs were then placed in test tubes filled with distilled water, with the lower portion of the bulb dipping in the water. The entire setup was kept at 28°C or higher until the roots reached a length of nearly 2 cm. The water in the test tubes was changed every 24 hours.

For 24 hours, the germinating bulbs with 2-3 cm long roots were placed over test tubes containing different concentrations of Ageratum treatment solutions. Bulbs were arranged so that only the roots were immersed in the test solutions. Following treatments, the bulbs were carefully removed from each treatment setup of both leaf and flower concentrations and thoroughly washed under running tap water. Each bulb's root tips were excised and fixed in Carnoy's fixative for 24 hours. The roots were transferred to rectified spirit after 24 hours of fixation and stored there until use.

Mitotic preparation was made by hydrolysing the root tips in a mixture of 1 N HCL and 2% acetocarmine (1:9) for 2 hours at 60+2 C. After that, they were heated intermittently for 5-10 minutes. Set aside for 20-30 minutes, covered. The root tip was then cut with a sharp blade and placed on a glass slide in a drop of 45 % glacial acetic acid before being covered with a cover slip. The tapping method squashed the root tip. Each root tip preparation was scanned, with 8-10 random cell observations under the microscope. The observation

included counting the number of dividing cells at various stages, calculating the mitotic index, and scoring a variety of cytological abnormalities.

For determining genotoxicity, the following parameters were used: (i) Mitotic index (MI), which was calculated as the percent ratio of dividing cells to total number of cells observed; (ii) Chromosome aberrations in onion cells, which were characterised and classified as disturbed metaphase, precocious movement, unorientation, laggard, stickiness, fragments, bridges, late separation, and so on.

Statistical analysis of data

The mean values for each concentration group and control were computed. The one-way ANOVA test (p0.05) was used to determine the significance of the means.

Results

The mitotic index (M1) was found to be maximum in the treated onion root tip cells under 10% concentration treatment in M1 generation, after which there was a gradual decrease in the mitotic index from lower to higher use dose it under both kinds of treatment leave as well as flower extract treatment some recovery occurred in M2 generation at all use dose it under both kinds of treatment but not up to the level of control except at 10% concentration treatment (Table 1, Fig. 1 a, b).

Chromosomal abnormalities at metaphase and anaphase

Microscopic examination of squashed *Allium cepa* root tip cells revealed that both extract treatments induced a number of mitotic abnormalities when compared to the control (Table 2 & 3, Fig – 2 & 3). The increasing doses of the extract in M1 generation caused an increase in mitotic abnormalities. Maximum chromosomal abnormalities were observed at the metaphase and anaphase stages under both types of treatment leaf extract (Table 2, Fig. 2 a, b) and flower extract (Table 3, Fig. 3 a, b). At Anaphase and Metaphase, the most common chromosomal abnormalities were disturbed metaphase (DM), precious moment (PM), laggard (LG), stickiness (SK), fragments (FR), bridges (BR), late separation (LS), and unequal separation (US).

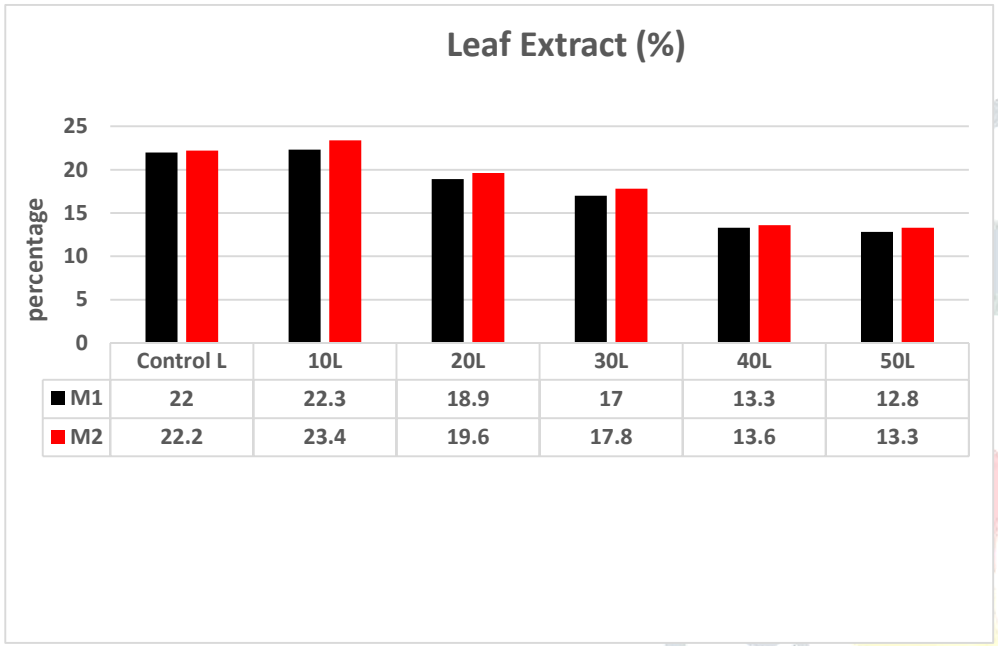
Except for the 10% concentration treatment, some recovery occurred in M2 generation at all used doses under both types of treatment, but not to the level of control (Table 2, 3; Fig. 2 a, b; 3 a, b).

Table: 1. **Effect of *Ageratum conyzoides* extracts on mitotic index of *Allium cepa* L.**

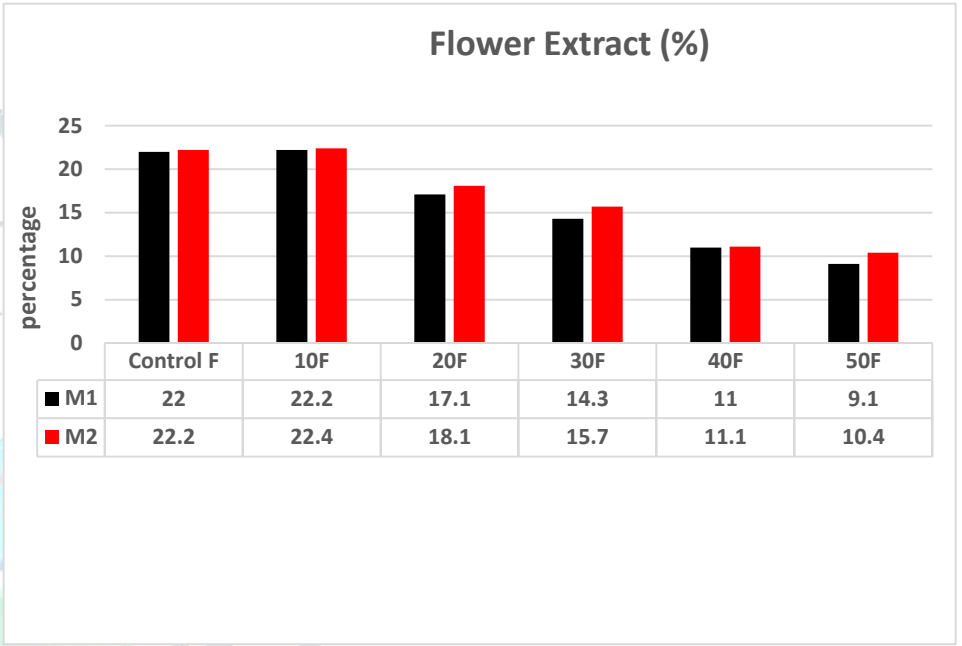
Treatment	Generation	Leaf Extracts			Flower Extracts		
		Total no. of cells observed	No. of actively dividing cell	Mitotic index (MI) Mean +SE	Total no. of cells observed	No. of actively dividing cell	Mitotic index (MI) Mean +SE
Control	**M1**	1512	334	**22.0 ± 1.06**	1512	334	**22.0 ± 1.06**
	M2	1518	338	**22.2 ± 1.06**	1518	338	**22.2 ± 1.06**
10	**M1**	1562	349	**22.3 ± 1.05**	1538	342	**22.2 ± 1.05**
	M2	1598	374	**23.4 ± 1.06**	1553	349	**22.4 ± 1.05**
20	**M1**	1497	283	**18.9 ± 1.01***	1456	249	**17.1 ± 0.98****
	M2	1592	312	**19.6 ± 0.99**	1509	274	**18.1 ± 0.99***
30	**M1**	1454	248	**17.0 ± 0.98***	1412	203	**14.3 ± 0.93****
	M2	1491	266	**17.8 ± 0.99****	1452	229	**15.7 ± 0.95****
40	**M1**	1372	183	**13.3 ± 0.91****	1334	148	**11.0 ± 0.85****
	M2	1448	198	**13.6 ± 0.90****	1373	153	**11.1 ± 0.84****
50	**M1**	1315	169	**12.8 ± 0.92****	1298	119	**09.1 ± 0.79****
	M2	1363	182	**13.3 ± 0.91****	1322	138	**10.4 ± 0.83****

****- Significant from the control at 1.00 % level.**

***- Significant from the control at 5.00 % level.**



(a)



(b)

Fig 1 a, b. Effect of leaf and flower extracts of *Ageratum conyzoides* L. on mitotic index of *Allium cepa* L.

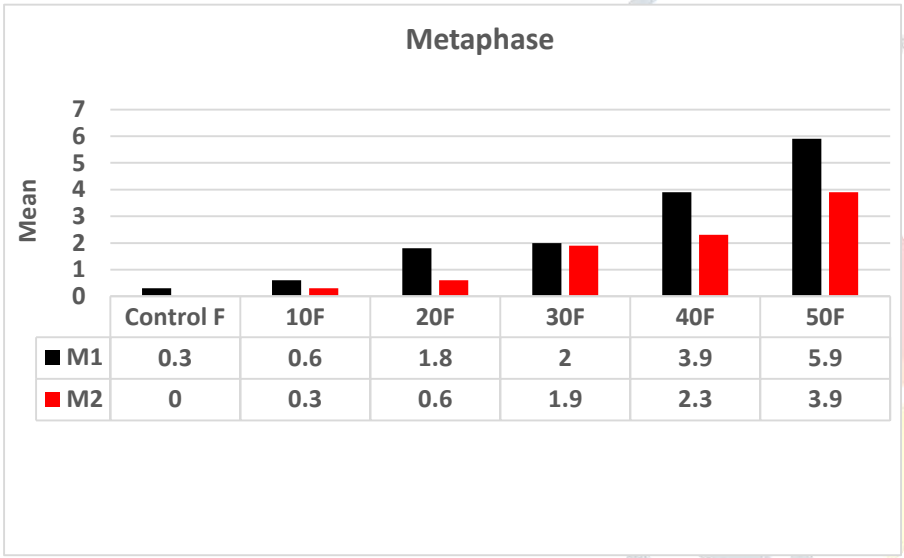
Table 2. Effect of leaf extract of *Ageratum conyzoides* L. on chromosomal abnormalities (%) in mitotically dividing cells of *Allium cepa* L.

Dose (%)	Generation	No of dividing cells	No of abnormal cells	Chromosomal abnormalities									
				Abnormalities at Metaphase			Total Mean ±SE	Abnormalities at Anaphase					Total Mean ±SE
				DM	PM	UN		LG	SK	FR	BR	LS	
Control	**M1**	334	01	-	0.30	-	**0.30 ± 0.30**	-	0.30	-	-	-	**0.30 ± 0.30**
	M2	338	01	-	-	-	**0.00 ± 0.00**	-	-	-	0.29	-	**0.30 ± 0.29**
10	**M1**	349	03	-	0.58	-	**0.60 ± 0.41**	-	-	0.29	-	-	**0.30 ± 0.29**
	M2	374	02	-	0.29	-	**0.30 ± 0.28**	-	-	-	-	-	**0.00 ± 0.00**
20	**M1**	283	10	0.72	0.72	0.35	**1.80 ± 0.79**	0.72	-	0.36	-	0.72	**1.80 ± 0.79**
	M2	312	07	-	-	0.65	**0.65 ± 0.45**	-	0.32	-	0.64	0.32	**1.30 ± 0.64**
30	**M1**	248	16	0.82	1.22	-	**2.04 ± 0.89**	0.82	-	0.82	1.21	1.21	**4.10 ± 1.25***
	M2	266	12	0.75	1.15	-	**1.90 ± 0.83***	0.38	1.15	-	0.75	-	**2.30 ± 0.91***
40	**M1**	183	19	1.11	1.65	1.10	**3.90 ± 1.43***	1.10	1.10	1.10	1.67	1.67	**6.64 ± 1.84****
	M2	198	15	-	1.15	1.15	**2.30 ± 1.06***	1.01	1.01	1.16	-	1.01	**4.20 ± 1.42***
50	**M1**	169	23	1.78	1.78	2.38	**5.94 ± 1.81****	1.79	1.79	1.79	1.79	1.19	**8.35 ± 2.12****
	M2	182	18	1.67	1.10	1.10	**3.90 ± 1.43***	1.11	1.11	0.56	1.66	2.21	**6.65 ± 1.84****

****- Significant from the control at 1.00 % level.**

***- Significant from the control at 5.00 % level.**

DM – disturbed Metaphase, PM – Precocious Movement, UN – Unorientation, LG – Laggard, SK – Stickiness, FR – Fragment, BR – Bridges, LS – Late Separation.



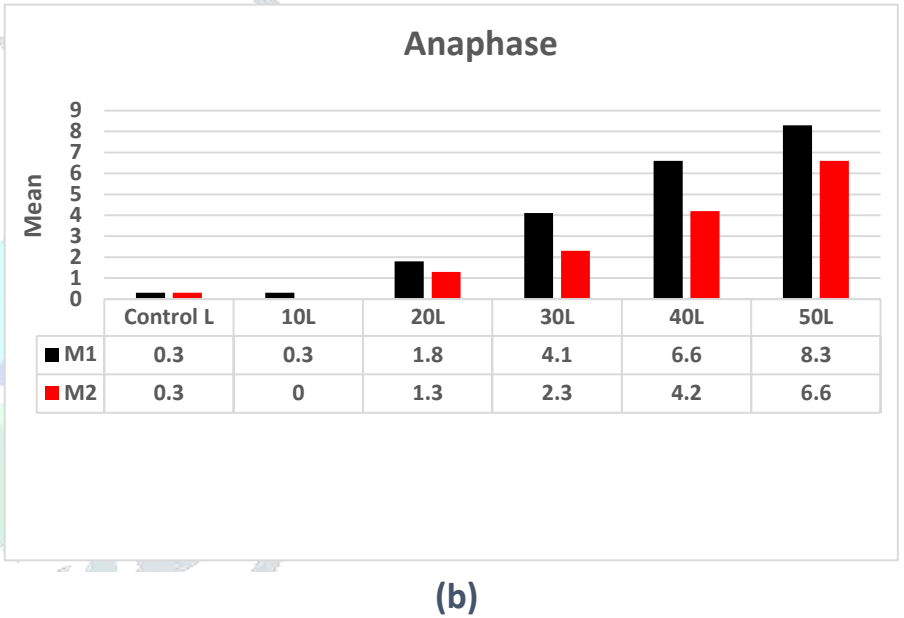


Fig - 2. a, b Effect of leaf extract of *Ageratum conyzoides* on chromosomal abnormalities (%) at Metaphase and Anaphase in mitotically dividing cells of *Allium cepa* L.

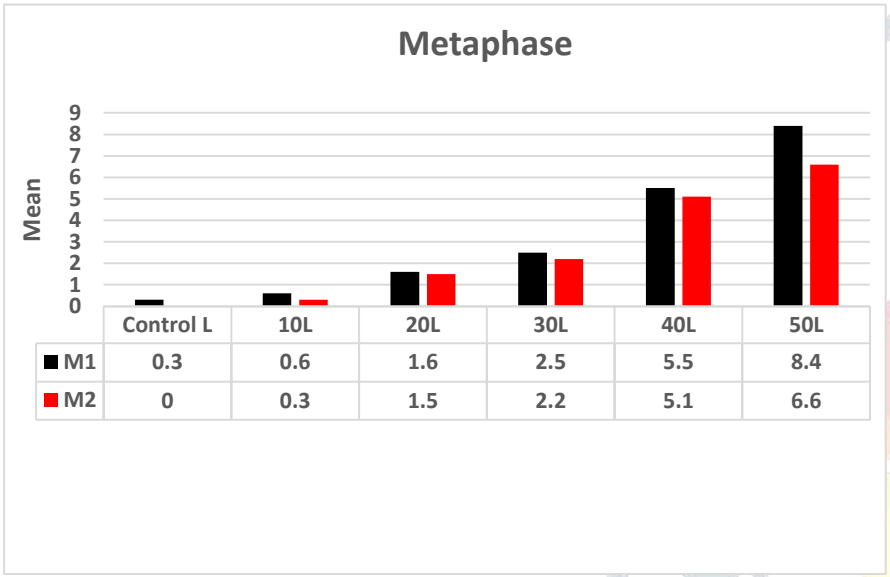
Table 3. Effect of flower extract of *Ageratum conyzoides* L. on chromosomal abnormalities (%) in mitotically dividing cells of *Allium cepa* L.

Dose (%)	Generation	No of dividing cells	No of abnormal cells	Chromosomal abnormalities									
				Abnormalities of Metaphase			Total Mean ±SE	Abnormalities of Anaphase					Total Mean ±SE
				DM	PM	UN		LG	SK	FR	BR	LS	
Control	**M1**	334	02	-	0.30	-	**0.30 ± 0.30**	-	-	-	-	-	**0.00 ± 0.00**
	M2	338	01	-	-	-	**0.00 ± 0.00**	-	-	-	-	0.29	**0.29 ± 0.30**
10	**M1**	342	04	0.59	-	-	**0.60 ± 0.41**	-	-	0.29	0.29	-	**0.60 ± 0.41**
	M2	349	02	-	0.29	-	**0.30 ± 0.30**	-	0.28	-	-	-	**0.30 ± 0.30**
20	**M1**	249	14	0.80	0.80	-	**1.60 ± 0.80**	0.81	0.81	1.20	0.81	-	**3.63 ± 1.18****
	M2	274	07	0.36	0.36	0.73	**1.50 ± 0.73***	-	-	-	0.37	0.73	**1.10 ± 0.63**
30	**M1**	203	17	1.49	0.99	-	**2.50 ± 1.09***	0.99	0.99	1.49	1.49	0.99	**5.95 ± 1.66****
	M2	229	14	0.89	0.44	0.89	**2.22 ± 0.97***	0.88	0.88	-	0.88	0.44	**3.08 ± 1.14***
40	**M1**	148	21	2.06	2.06	1.37	**5.50 ± 1.87***	1.37	2.06	1.37	2.06	2.06	**8.92 ± 2.34****
	M2	153	19	1.32	1.32	1.97	**5.10 ± 1.77***	1.32	1.97	1.97	1.32	1.32	**7.90 ± 2.18****
50	**M1**	119	26	2.52	3.36	2.52	**8.40 ± 2.54****	2.53	2.53	3.35	2.53	1.68	**12.62 ± 3.04****
	M2	138	22	2.18	2.18	2.18	**6.60 ± 2.11****	1.46	2.19	1.46	2.91	2.19	**10.21 ± 2.57****

****- Significant from the control at 1.00 % level.**

***- Significant from the control at 5.00 % level.**

DM – disturbed Metaphase, PM – Precocious Movement, UN – Unorientation, LG – Laggard, SK – Stickiness, FR – Fragment, BR – Bridges, LS – Late Separation.



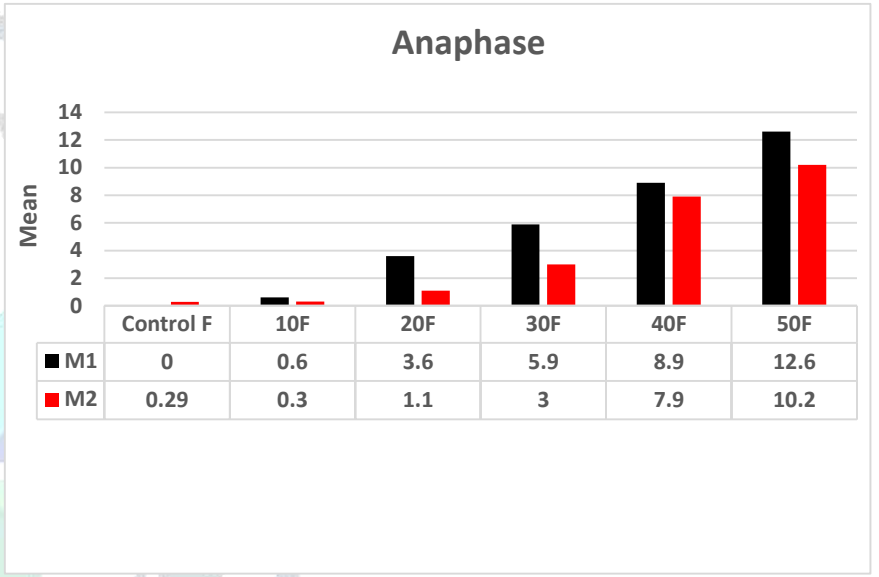


Fig - 3. a, b Effect of flower extract of *Ageratum conyzoides* on chromosomal abnormalities (%) at Metaphase and Anaphase in mitotically dividing cells of *Allium cepa* L.

Discussion

The allium test is more sensitive, standard, and widely used. According to **[4] [12]**, the Allium test is widely used to assess the quality of drinking water and environmental pollution. According to Ma **[10]**, the International Programme on Plant Bioassay (IPPB) has adopted the Allium root tip chromosomal aberration assay for monitoring environmental pollutants. There are several advantages to using *Allium cepa* for genotoxicity testing: (1) The mitotic phase is very clear; (2) the chromosome morphology is diverse; (3) the number of chromosomes is low (2n=16); and (4) the response to the genotoxic substance is clear and fast.

The current study found that aqueous leaf and flower extracts of *Ageratum conyzoide* had a negative effect on the root tip of *Allium cepa*, disrupting the genetic make-up. In both types of treatment, there was a gradual decrease in mitotic index from lower to higher used doses in M1 generation. The control plants had the highest percentage of mitotically dividing cells, while the % concentration treatment had the lowest. The frequency of abnormality mitotically diving cell increased stepwise with increasing doses due to the deleterious effect of *Ageratum conyzoide* extracts. It included a number of abnormalities such as disturbed metaphase, precocious movement of chromosomes, chromosome orientation at metaphase stage, Laggards, bridge formation, stickiness, fragmentation, and late separation of chromosomes at anaphase stage.

There were no abnormalities in the M2 generation at 10% concentration. During the anaphase stage, aqueous leaf and flower extracts of Ageratum weed had a significant effect on chromosomal behaviour. According to **[14],** anaphase chromatin bridges are formed as a result of unequal exchanges, resulting in the formation of dicentric chromosomes that are pulled equally to both poles during anaphase.

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