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Research Article

ALLELOPATHIC AND MITODEPPRESSIVE EFFECTS OF AQUEOUS EXTRACTS OF *LANTANA CAMARA* L. ON *VIGNA UNGUICULATA* (L.) WALP. CV. BCP-25

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ABSTRACT
Lantana camara L. (Verbenaceae) grown as ornamental shrubs is a major exotic weed spreading rapidly in wastelands and agricultural fields. The ability of this weed to germinate fast and to inhibit the growth of other neighbouring plants paved way for its quicker growth in an ecosystem. The allelochemicals of the weed have contributed towards its dominance over the crop plants too. Because of the extraordinary spread, dominance and its naturalisation in India in a short period of time, this weed was taken to assess the allelopathic effects and cytotoxic activities of aqueous extracts of its root, stem, leaf, flower and fruit on the seven day old seedlings *Vigna unguiculata* (L) Walp. cv. BCP-25, one of the most important tropical dual-purpose legumes used as grain as well as fodder. The LD_{50} concentration for the root and leaf extracts was recorded as 26% and 285 respectively, while 31% concentration for flower and 32% for stem and fruit extracts proved to be $LD_{50.}$ The extracts from all the parts of the weed decreased the mitotic index of cowpea root tips with increasing concentrations (5, 10, 15 and 20%). However the chromosomal abnormalities increased rapidly, the highest being with root extract (25.14%), followed by leaf (16.47%), flower (11.69%), stem (10.12%) and fruit (9.12%) at 20% concentration. Seven types of chromosomal aberrations *viz.*, fragments, stickiness, micronuclei, laggards and bridges were observed in all extract applications. The allelochemicals in the root and leaves of *Lantana camara* L. were highly carcinogenic and served as a potent tool in eroding the chromosomes of cowpea. This study warns that the weed leachates have been proved to be dangerously mitodepressive and so high priority be given to prevent this weed from further intrusion into cultivable land.

Keywords: *Lantana Camara L., Root, Stem, Leaf, Flower and Fruit Extracts, Cowpea, Allelopathic Effects, Cytotoxicity*

INTRODUCTION
Over the last many decades, a number of Forest Invasive Species (FIS), without realizing the consequences, have been introduced in India. *Lantana camara* L. is one such invasive weed causing disturbance to the native composition of terrestrial ecosystem (Rajendiran, 1999a). Allelochemics, the non-nutritional secondary metabolites were produced by these weeds, which were liberated by volatilization from aerial parts, exudation from roots, leaching from plants and their residues by rain or by decomposition of residues (Nikki and Scott, 2010). Allelochemicals have a wide mode of action and the quantity and concentration of such chemical compounds released into the environment by a species is directly responsible for the survival as well as dominance of that species and reduction or even elimination of associated plant species (Aneja *et al.*, 1991; Rajendiran, 2000a,c; Bertholdsson, 2012; Hridya and Rajendiran, 2014). *Vigna unguiculata* (L) Walp. cv. BCP-25 used as test plant in this study is an important and commercially popular legume in India. Even though several works of allelopathy have been done with various crop plants, only little work on cytotoxity of allelochemics in legumes have been carried out. Hence, it was thought worthwhile to evaluate the influence of aqueous extracts of root, stem, leaf, flower and fruit of the weed *Lantana camara* L. on seedling establishment and cell division of *Vigna unguiculata* (L) Walp. cv. BCP-25 one of the most important tropical dual-purpose legume, being used for vegetables (leaves and flowers), grain, as fresh cut and carry forage and for hay and silage.

MATERIALS AND METHODS
The certified seeds of cowpea (*Vigna unguiculata* (L) Walp. cv. BCP-25) were obtained from Department

of Vegetable crops, Tamil Nadu Agricultural University, Coimbatore. The fresh root, stem, leaf, flower and fruit of *Lantana camara* L. collected from Pondicherry, were washed and ground separately in an electric grinder and the extracts were prepared in each case by boiling 10 gm of ground plant material in 100 ml of distilled water at 100^{o}C for 25 minutes. After filtration with Whatman No.1 filter paper, stock solutions were prepared.

For determining the LD_{50} concentration of the four extracts, three separate sets of experiments each with triplicates were carried out and the data presented in Table 1. In the first set, various concentrations of root, stem, leaf, flower and fruit extracts (25, 50, 75, and 100%) of *Lantana camara* L. were made in distilled water. Viable seeds of cowpea (*Vigna unguiculata* (L) Walp. cv. BCP-25), soaked in distilled water for 6 hours were allowed to germinate in petri plates lined with moist Whatman No.1 filter paper. Seven days old seedlings with healthy roots were chosen to accommodate 25 seedlings in a petriplate for each treatment. The healthy seedlings were treated separately with 5 ml of each concentration of the extracts for three days. Seedlings watered with distilled water served as control. The second treatment of different concentrations of the weed extracts (25, 30, 35, 40, 45, 50% concentrations) was given to fresh set of seedlings grown in petri plates. The third set of treatment consisted of 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35% concentrations of the weed extracts to a new set of seven day old seedlings.

The root tips of 10 day old seedlings were highly injured after treatment with 25 to 35% concentrations of the weed extracts. Even though few seedlings survived their root tips were unhealthy for preparing root tip squash as they developed scars in response to the injuries caused by the extract treatments. Hence the cytological studies with three test plants were restricted to 5, 10, 15 and 20% concentrations of the weed extracts. The root tips were excised from the control and treated seedlings (5, 10, 15 and 20% concentrations of the five extracts) after three days of extract treatment, washed in distilled water and fixed in Carnoy's fixative for 24 hours. Root tip squash technique of Rajendiran (2005) was followed. The mitotic index in control and treated root tip cells were calculated. The prepared slides were thoroughly examined for the presence of different types of chromosomal aberrations, important stages photographed in Labomed Photo Microscope and the data recorded.

RESULTS AND DISCUSSION

The root, stem, leaf, flower and fruit extracts of *Lantana camara* L. affected the process of seedling growth in *Vigna unguiculata* (L) Walp. cv. BCP-25. All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 23.33 to 43.33% in 25% concentration of all the extracts (Table 1). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the root and leaf extracts died, while all the seedlings treated with 40, 45, 50% concentrations of the stem, flower and fruit extracts died. In 25, 30 and 35% concentrations the lethality ranged from 23.33 to 83.33% (Table 1). In the third set of experiments the LD_{50} concentration for the root and leaf extracts was recorded as 26%, for flower extract it was 31%, while 32% concentrations of stem and fruit extracts proved to be LD_{50} (Table 1). The maximum inhibition of seedling growth was recorded at the highest concentration of root extract treatment. From the collected data it is evident that differential effect of the extracts on seedling growth revealed the presence of highest concentration of inhibitory allelochemics in the root of the weed followed by leaf, flower, stem and fruit. Rajendiran (1999a) in *Helianthus annuus* L. seedlings has reported similar inhibitions of seedling growth by this weed extracts.

In control condition, the root tips of *Vigna unguiculata* (L) Walp. cv. BCP-25 showed normal cell division (Plate 1, Figure 1). Mitotic index of *Vigna unguiculata* (L) Walp. cv. BCP-25 showed a steady decrease with increasing concentrations of all the extracts (Table 2). The percentage value of mitotic index in control was 33.33% and after treatment with root, stem, leaf, flower and fruit extracts it declined rapidly with the increase in concentrations. The least values of 8.71%, 16.77%, 11.57%, 14.44% and 15.46% were recorded after treatment with root, stem, leaf, flower and fruit extracts respectively in 20% concentration (Table 2; Plate 1, 2). Similar observations were reported in *Ammi majus* (Adam and Rashad, 1984), *Datura stramonium* (Rajendiran, 1996), *Azadirachata indica* (Rajendiran, 1998a), *Catharanthus roseus* (Rajendiran, 1998b), *Lantana camara* (Rajendiran, 1999a), *Ricinus communis*

(Rajendiran, 1999b), *Adhatoda vasica* (Rajendiran, 1999c), *Boerhaavia diffusa* (Rajendiran, 2000b) and in *Parthenium hysterophorus* L. extracts (Hridya and Rajendiran, 2013a,b,c, 2014). All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 2; Plate 1, 2). However, the extracts of root and leaf caused severe inhibition and greater number of chromosomal abnormalities (25.14 and 16.47% respectively) than the flower (11.69%), stem (10.12%) and fruit (9.12%) extracts at 20% concentration, the least being with fruit extract (Table 2; Plate 1, 2). Application of extracts of the weed changed the normal cycle of events of mitosis in *Vigna unguiculata* (L) Walp. cv. BCP-25 root tip cells producing chromosome fragments (Plate 1, Figure 2), stickiness of chromosome ends (Plate 1, Figure 3), ring chromosomes (Plate 1, Figure 4), bridge formation and (Plate 1, Figure 5) laggards formation (Plate 1, Figure 6) at anaphase and telophase, micronuclei (Plate 1, Figure 7) and polyploidy cell (Plate 1, Figure 8).

Table 1: Lethality of the leaf, stem, root, flower and fruit extracts of *Lantana camara* L. on the 7 day old seedlings of *Vigna unguiculata* (L) Walp. cv. BCP-25 after 3 days of treatment

Expt. Set No.	Extract Concentration	Root (%)	Stem (%)	Leaf (%)	Flower (%)	Fruit %)
1	**25 %**	43.33	28.33	41.66	33.33	23.33
	50 %	100	100	100	100	100
	75 %	100	100	100	100	100
	100 %	100	100	100	100	100
Expt. Set No.	**Extract Concentration**	**Root (%)**	**Stem (%)**	**Leaf (%)**	**Flower (%)**	**Fruit %)**
2	**25 %**	43.33	28.33	41.66	33.33	23.33
	30 %	58.33	41.66	53.33	46.66	33.33
	35 %	100	83.33	100	78.33	76.66
	40 %	100	100	100	100	100
	45 %	100	100	100	100	100
	50 %	100	100	100	100	100
Expt. Set No.	**Extract Concentration**	**Root (%)**	**Stem (%)**	**Leaf (%)**	**Flower (%)**	**Fruit %)**
3	**25 %**	43.33	28.33	41.66	33.33	23.33
	26 %	50	31.66	50	35.00	26.66
	27 %	51.66	33.33	51.66	38.33	26.66
	28 %	55.00	38.33	51.66	43.33	28.33
	29 %	56.66	38.33	53.33	45.00	31.66
	30 %	58.33	41.66	53.33	46.66	33.33
	31 %	63.33	45.00	58.33	50	40.00
	32 %	71.66	50	70.00	63.33	50
	33 %	88.33	60.00	85.00	76.66	60.00
	34 %	95.00	78.33	93.33	85.00	78.33
	35 %	100	83.33	100	78.33	76.66

The root and leaf of the weed showed severe inhibitory effects and were extremely clastogenic and spindle poisoning when compared with the extracts of the stem and root. This result correlated with the report of Singh *et al.* (1983a, b) that the toxins *viz.* Lantadane-A and Lantadane-B were maximum in the root and leaf of *Lantana camara* L. followed by flower, stem and fruit. These two toxic principles from the weed induced changes in macromolecules, proteins, nucleic acids and lipids which manifested in massive damage to cellular membranes and loss of enzyme activity (Rajendiran 1999a). Lantadane-A reacted with many proteins, while Lantadane-B is the novel uncouplers of oxidative phosphorylation (Rajendiran 1999a). Due to non-availability of the required enzymes to support DNA replication and protein deficiency reducing the production of histones, abnormal cell divisions with aberrated chromosomes were formed (Rajendiran 2000c). The present study with *Vigna unguiculata* (L) Walp. cv. BCP-25 deemntrated that the root and leaf of *Lantana camara* L. played the role of maintaining the dominance of the weed by poisoning the mitotic apparatus of other plant species. Immediate action to prevent this weed from spreading further is required as the aqueous extracts of *Lantana camara L.* have proved to be highly effective in wearing away the chromosomes of cowpea.

Table 2: Mitosis and chromosomal aberrations induced by *Lantana camara* L. extracts in *Vigna unguiculata* (L) Walp. cv. BCP-25 root tip cells

Extract	Conc. (%)	Dividing cells (%)	Abnormal cells (%)	Stickiness (%)	Laggards (%)	Bridge (%)	Chromosome breakage (%)	Polyploidy (%)	Micronuclei (%)	Ring chromosomes (%)
Control		33.33	-	-	-	-	-	-	-	-
Root	5	14.52	9.44	2.81	2.12	1.95	1.57	0.99	-	-
	10	10.60	14.11	3.61	2.67	2.43	2.03	1.78	0.93	0.66
	15	9.69	19.22	4.48	3.48	3.22	2.87	2.23	1.74	1.20
	20	8.71	25.14	6.32	4.25	4.18	3.51	3.01	2.53	1.34
Stem	5	21.02	2.87	1.33	1.54	-	-	-	-	-
	10	19.82	5.46	2.48	1.16	0.95	0.87	-	-	-
	15	17.48	8.84	2.22	1.73	1.57	1.30	0.67	-	-
	20	16.77	10.12	2.97	2.25	2.08	1.70	1.12	-	-
Leaf	5	17.23	4.79	1.79	1.11	0.89	0.91	-	-	-
	10	15.59	7.42	2.28	1.69	1.36	1.23	0.86	-	-
	15	13.18	11.24	3.18	2.19	1.91	1.79	1.32	0.38	0.38
	20	11.57	16.47	4.08	3.00	3.09	2.36	1.97	0.90	1.07
Flower	5	19.69	2.90	1.44	0.73	0.73	-	-	-	-
	10	18.78	6.57	2.58	1.33	1.17	1.00	0.38	-	-
	15	16.74	9.14	2.44	1.88	1.69	1.38	0.78	0.39	0.60
	20	14.44	11.69	3.03	2.64	2.19	1.78	1.38	0.67	-
Fruit	5	20.07	2.76	1.23	1.53	-	-	-	-	-
	10	18.73	5.57	2.49	1.19	0.97	0.92	-	-	-
	15	16.98	8.64	2.22	1.63	1.47	1.30	0.67	-	-
	20	15.46	9.12	2.57	2.05	2.08	1.30	1.12	-	-

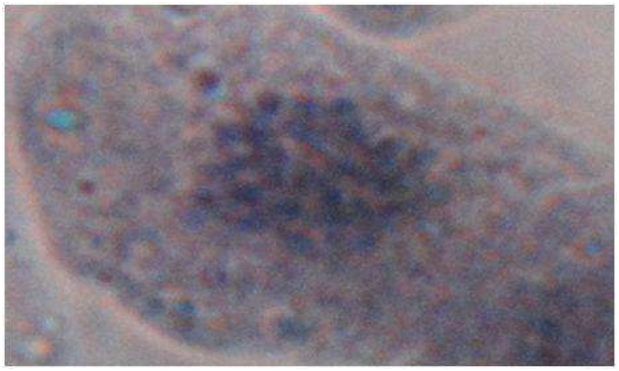

Figure 1: Normal somatic metaphase (2n=22)

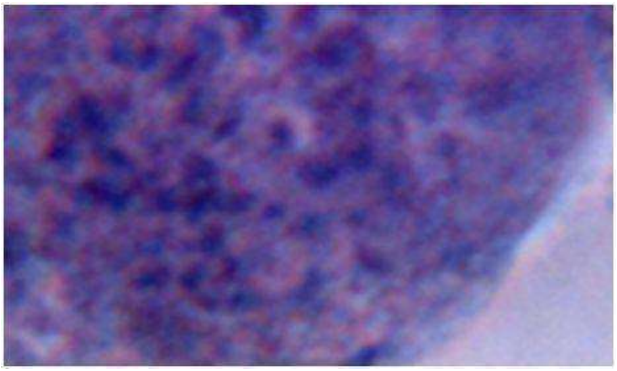

Figure 2: Chromosome fragments

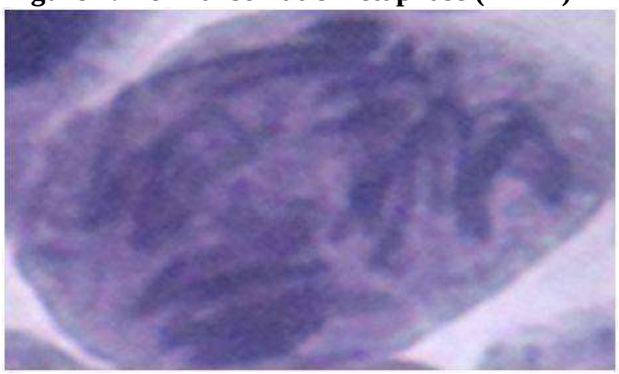

Figure 3: Stickiness of chromosome ends

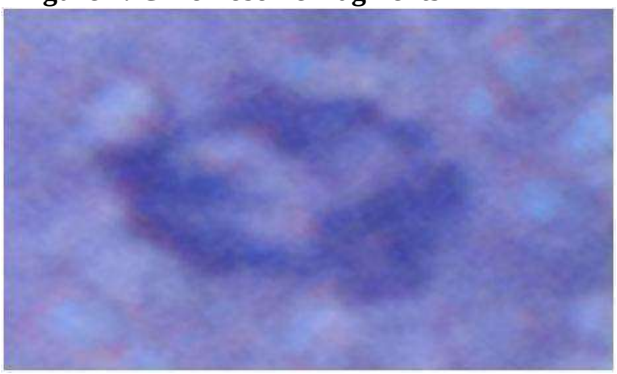

Figure 4: Ring chromosomes

Figure 5: Chromosome bridges

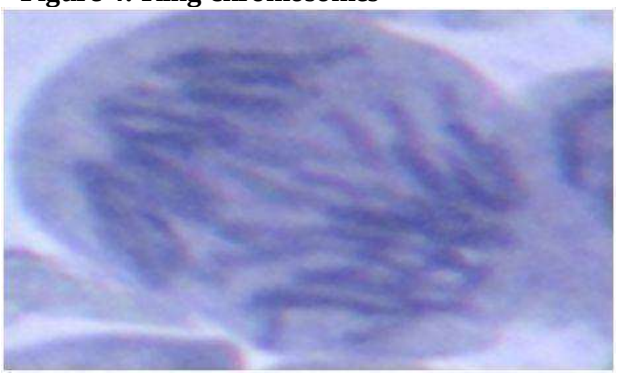

Figure 6: Anaphasic laggards

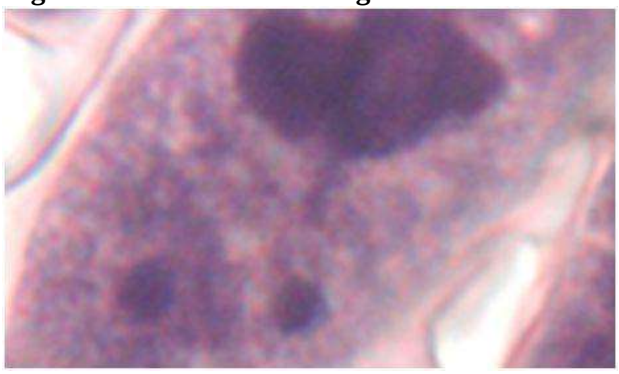

Figure 7: Micronuclei

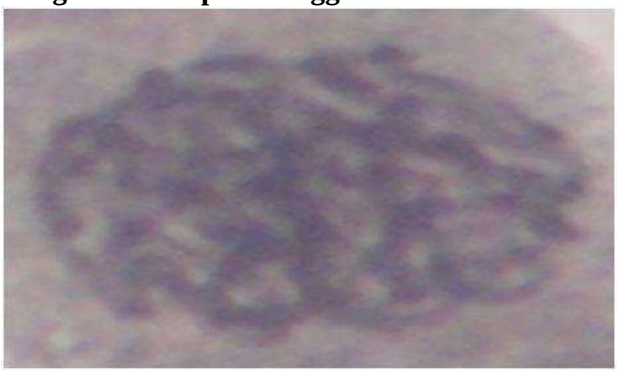

Figure 8: Polyploidy

Plate 1: Somatic metaphase and chromosomal abnormalities induced by *Lantana camara* L. extracts in the root tip cells of *Vigna unguiculata* (L) Walp. cv. BCP-25
(Figs. 1-8: 1000x)

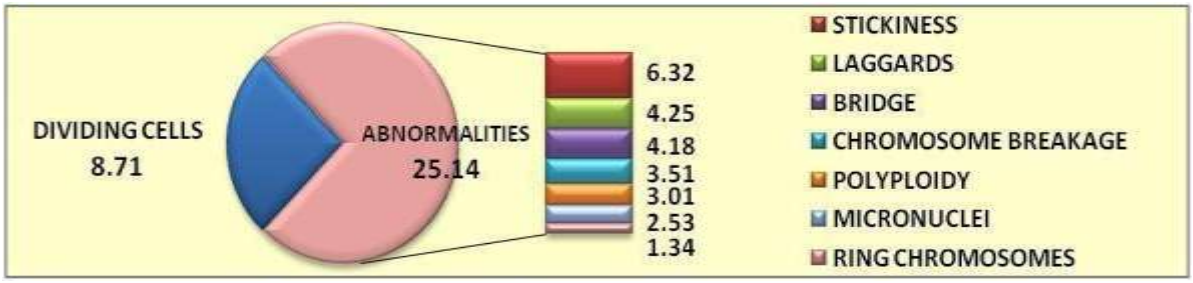

Figure 1: Root extract

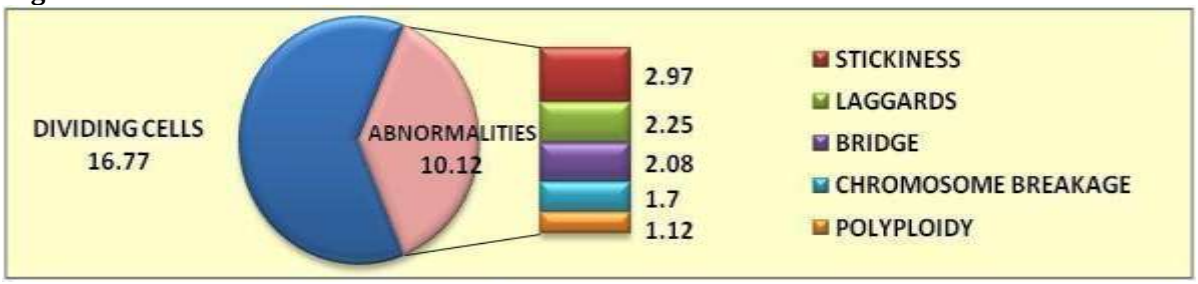

Figure 2: Stem extract

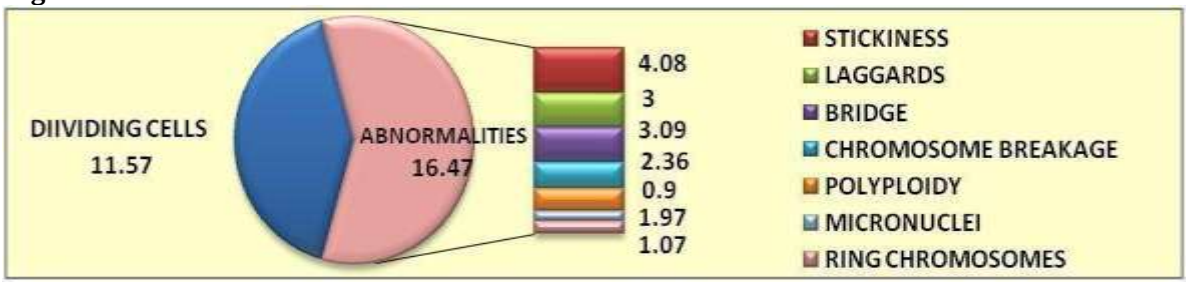

Figure 3: Leaf extract

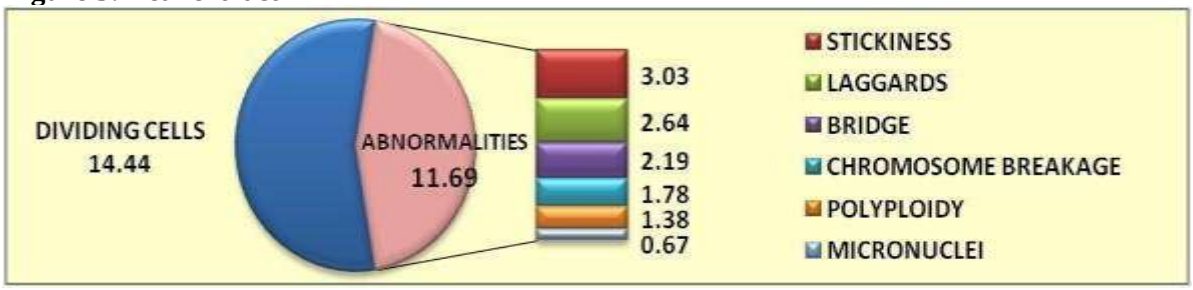

Figure 4: Flower extract

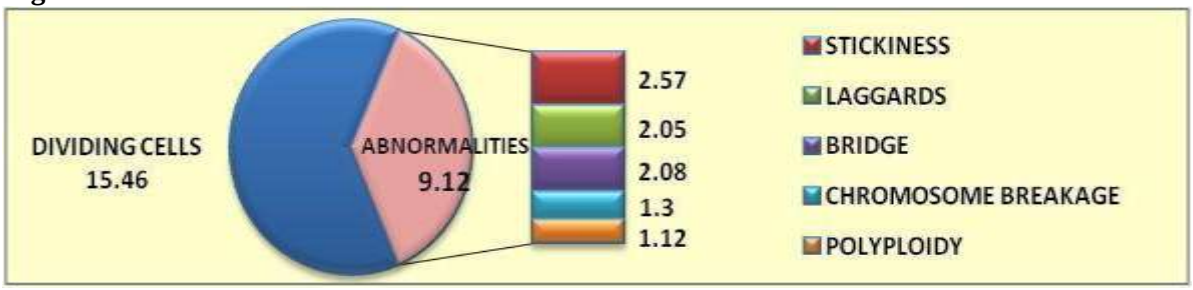

Figure 5: Fruit extract

Plate 2: Mitotic divisions, chromosomal abnormalities and their types induced by 20% concentrations of weed extracts in *Vigna unguiculata* (L) Walp. cv. BCP-25

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