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Research Article

ALLELOPATHIC AND CYTOTOXIC EFFECTS OF AQUEOUS EXTRACTS OF *LANTANA CAMARA* L. ON *VIGNA MUNGO* L. VAR. VAMBAN-16

***K. Rajendiran, D. Yogeswari and D. Arulmozhi**
Department of Botany, K.M. Centre for Post Graduate Studies, Pondicherry – 605 008
**Author for Correspondence*

ABSTRACT
Lantana (*Lantana camara* L., Verbenaceae) is a shrub that was grown as ornamentals and is now major exotic weed disturbing the native composition of terrestrial ecosystem. The toxic allelochemicals present in this weed have contributed towards its dominance over the neighbouring plants including crops. Owing to the extraordinary spread, dominance and its naturalisation in India in a short 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 of *Vigna mungo* L. var. Vamban-16, one of the most important legumes, being used for grain. The LD_{50} concentration for the leaf and root extracts was recorded as 26%, 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 black gram root tips with increasing concentrations (5, 10, 15 and 20%). However the chromosomal abnormalities increased rapidly, the highest being with root extract (24.75%), followed by leaf (17.55%), flower (13.70%), stem (10.56%) and fruit (9.93%) 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 black gram. The present work cautions that the weed leachates have been proved to be dangerously mitodepressive and so the prevention of this weed from further intrusion into cultivable land is urgent.

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

INTRODUCTION
Invasive alien species (IAS) are the greatest threat to biodiversity around the globe. The introduction of IAS can be intentional or accidental but they can affect the structure and function of ecosystems. *Lantana camara* L. is one such invasive terrestrial weed, causing huge repercussions to the native composition of terrestrial ecosystem (Rajendiran, 1999a). These weeds liberate allelochemics (non-nutritional secondary metabolites) 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 mungo* L. var. Vamban-16 used as test plant in this study is an important and commercially popular cereal in India. Even though several works of allelopathy have been done with various crop plants, only little work on cytotoxity of allelochemics in cereals have been carried out. Hence, it was sufficiently important to study the influence of aqueous extracts of root, stem, leaf, flower and fruit of *Lantana camara* L. on seedling establishment and cell division of an important legume, *Vigna mungo* L. var. Vamban-16.

MATERIALS AND METHODS
The certified seeds of black gram (*Vigna mungo* L. var. Vamban-16) 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 black gram (*Vigna mungo* L. var. Vamban-16), 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.

After treatment with 25 to 35% concentrations of the weed extracts the root tips of 10 day old seedlings were highly injured. 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.

RESULT AND DISCUSSION

The root, stem, leaf, flower and fruit extracts of *Lantana camara* L. affected the process of seedling growth in *Vigna mungo* L. var. Vamban-16. All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 21.66 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 21.66 to 81.66% (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 by *Lantana camara* L. extracts.

In control condition, the root tips of *Vigna mungo* L. var. Vamban-16 showed normal cell division (Plate 1, Figure 1). Mitotic index of *Vigna mungo* L. var. Vamban-16 showed a steady decrease with increasing concentrations of all the extracts (Table 2). The percentage value of mitotic index in control was 42.46% and after treatment with root, stem, leaf, flower and fruit extracts it declined rapidly with the increase in concentrations. The lowest values of 11.71%, 19.88%, 14.57%, 17.44% and 18.46% were recorded for the mitotic divisions 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 (25.93 and 17.85 % respectively) than the flower (14.13), stem (11.11) and fruit (10.22) extracts at 20% concentration, the least being with fruit extract (Table 2; Plate 1, 2). The normal cycle of events of mitosis in *Vigna mungo* L. var. Vamban-16 root tip cells was changed after application of weed extracts which produced chromosome fragments (Plate 1, Figure 2), stickiness of chromosome ends (Plate 1, Figure 3), ring chromosomes (Plate 1, Figure 4), chromosome bridges (Plate 1, Figure 5), laggard formation (Plate 1, Figure 6), micronuclei (Plate 1, Figure 7) and polyploidy (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 mungo* L. var. Vamban-16 after 3 days of treatment

Expt. Set No.	Extract Concentration	Root (%)	Stem (%)	Leaf (%)	Flower (%)	Fruit (%)
1	25 %	43.33	26.66	41.66	30.00	21.66
	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	26.66	41.66	30.00	21.66
	30 %	56.66	40.00	53.33	45.00	31.66
	35 %	100	81.66	100	81.66	73.33
	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	26.66	41.66	30.00	21.66
	26 %	50	28.33	45.00	35.00	23.33
	27 %	51.66	31.66	48.33	38.33	25.00
	28 %	53.33	33.33	50	41.66	28.33
	29 %	55.00	36.66	51.66	43.33	30.00
	30 %	56.66	40.00	53.33	45.00	31.66
	31 %	65.00	43.33	61.33	50	43.33
	32 %	75.00	50	73.33	61.66	50
	33 %	85.00	58.33	90.00	75.00	60.00
	34 %	95.00	78.33	93.33	78.33	71.66
	35 %	100	81.66	100	81.66	71.33

The root and leaf of the weed showed severe inhibitory effects and were extremely genotoxic 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 study with *Vigna mungo* L. var. Vamban-16 disclosed that the root and leaf of *Lantana camara* L. were severe genotoxic agents playing the major role in maintaining the dominance of the weed by inhibiting the growth of surrounding plants. Hence immediate action to control this weed is required as the aqueous extracts of *Lantana camara L*. have proved to be potent enough to destroy the genome of black gram.

Table 2: Mitosis and chromosomal aberrations induced by *Lantana camara* L. extracts in *Vigna mungo* L. var. Vamban-16 root tip cells

Extract	Conc. (%)	Dividing cells (%)	Abnormal cells (%)	Stickiness (%)	Laggards (%)	Bridge (%)	Chromosome breakage (%)	Polyploidy (%)	Micronuclei (%)	Ring chromosomes (%)
Control		42.46	-	-	-	-	-	-	-	-
Root	5	17.52	9.75	2.92	2.23	1.96	1.68	0.96	-	-
	10	13.60	14.80	3.72	2.78	2.54	2.14	1.89	0.96	0.77
	15	12.69	19.99	4.59	3.59	3.33	2.98	2.34	1.85	1.31
	20	11.71	25.93	6.43	4.36	4.29	3.62	3.12	2.64	1.47
Stem	5	24.02	2.90	1.45	1.45	-	-	-	-	-
	10	22.82	5.64	2.59	1.27	0.89	0.89	-	-	-
	15	20.48	8.23	2.44	1.87	1.69	1.45	0.78	-	-
	20	19.88	11.11	2.98	2.67	2.28	1.78	1.45	-	-
Leaf	5	20.23	4.74	1.67	1.22	0.87	0.98	-	-	-
	10	18.59	7.66	2.39	1.70	1.47	1.32	0.78	-	-
	15	16.18	11.87	3.56	2.34	1.99	1.97	1.33	0.34	0.34
	20	14.57	17.85	4.56	3.23	3.23	2.57	1.78	0.78	1.70
Flower	5	22.69	2.79	1.33	0.73	0.73	-	-	-	-
	10	21.78	7.02	2.67	1.22	1.34	1.23	0.56	-	-
	15	19.74	9.80	2.34	1.56	1.78	1.89	0.56	0.78	0.89
	20	17.44	14.13	3.78	2.67	2.89	1.45	1.67	1.67	-
Fruit	5	23.07	3.24	1.56	1.68	-	-	-	-	-
	10	21.73	5.75	2.56	1.23	0.98	0.98	-	-	-
	15	19.98	8.46	2.56	1.67	1.78	1.78	0.67	-	-
	20	18.46	10.22	2.67	2.55	2.55	0.67	1.78	-	-

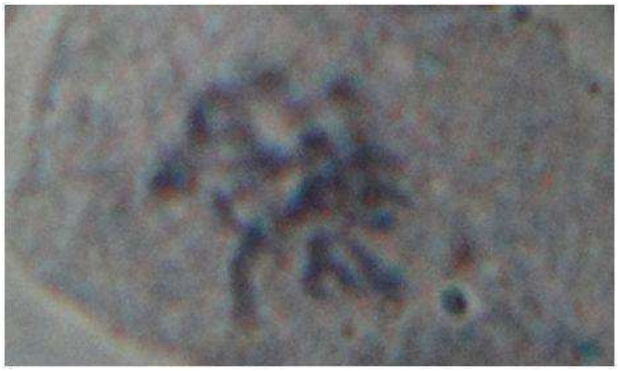

Figure 1: Normal somatic metaphase (2n=22)

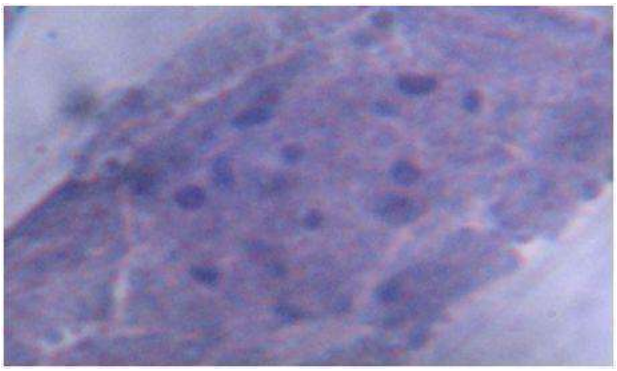

Figure 2: Chromosome fragments

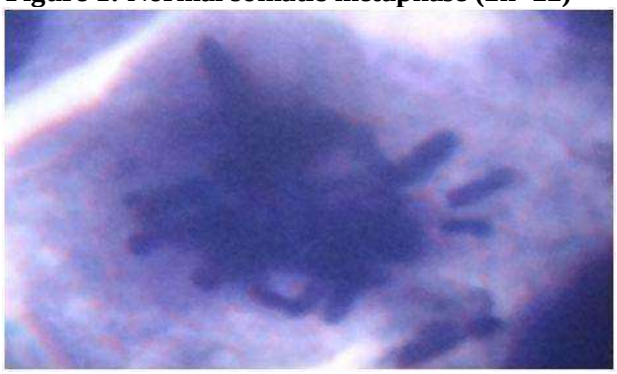

Figure 3: Stickiness of chromosomes

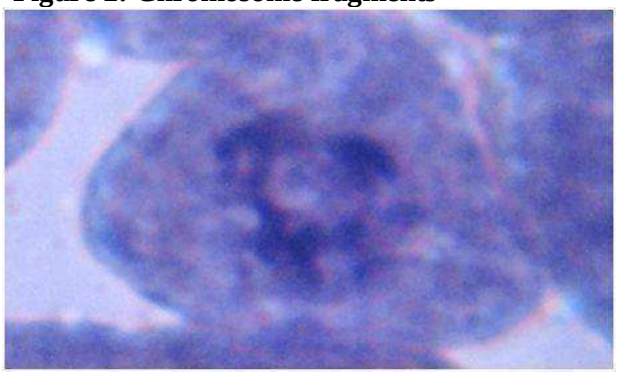

Figure 4: Ring chromosomes

Figure 5: Anaphasic bridges

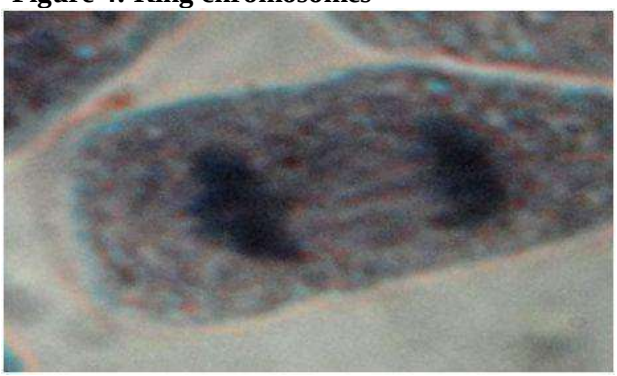

Figure 6: Telophasic laggard

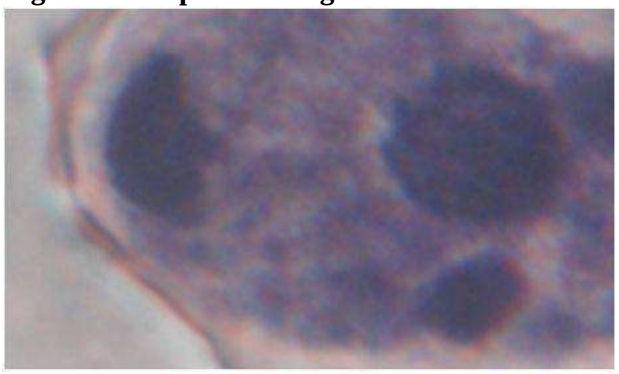

Figure 7: Micronuclei

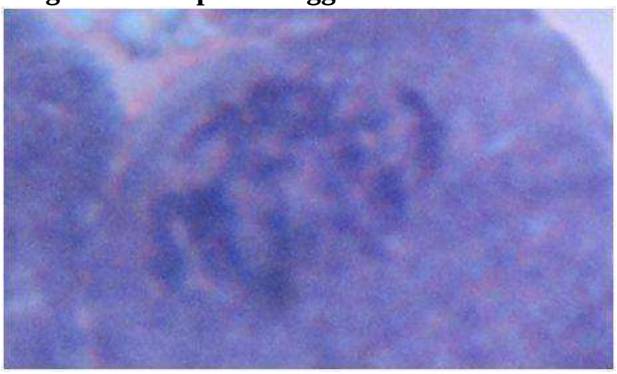

Figure 8: Polyploid cell

Plate 1: Somatic metaphase and chromosomal abnormalities induced by *Lantana camara* L. extracts in the root tip cells of *Vigna mungo* L. var. Vamban-16
(Figs. 1-8: 1000x)

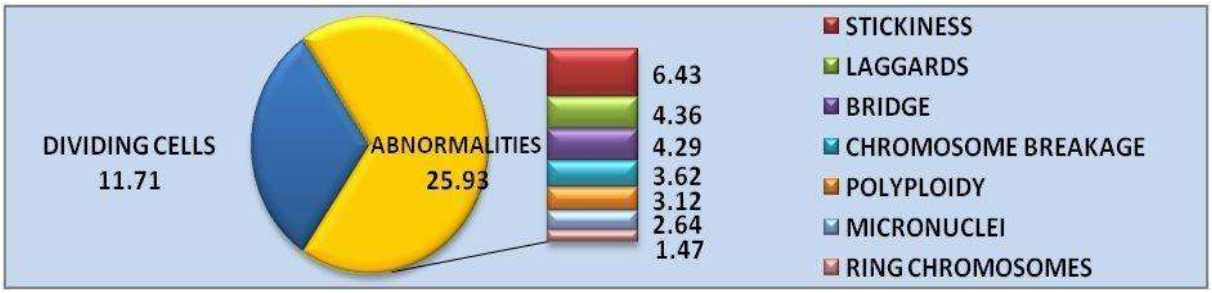

Figure 1: Root extract

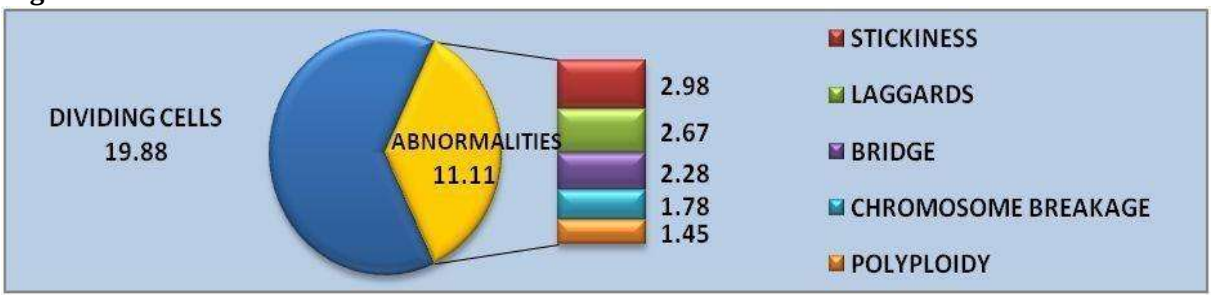

Figure 2: Stem extract

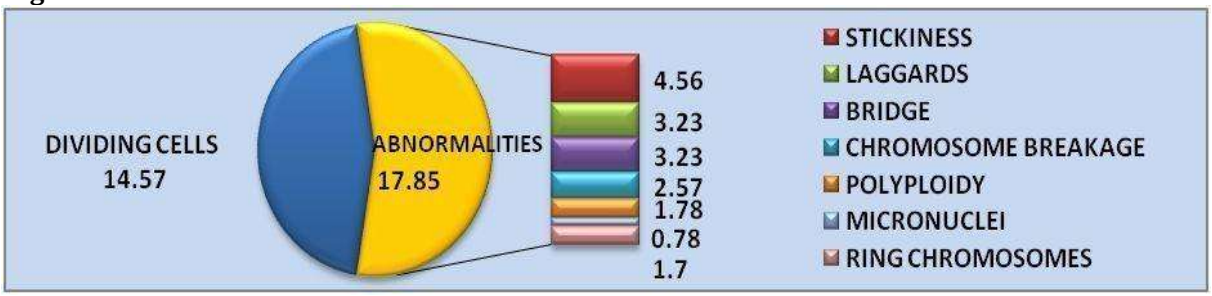

Figure 3: Leaf extract

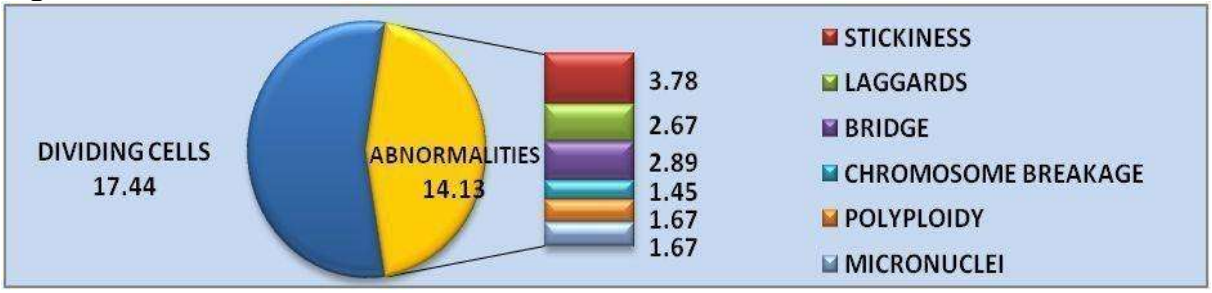

Figure 4: Flower extract

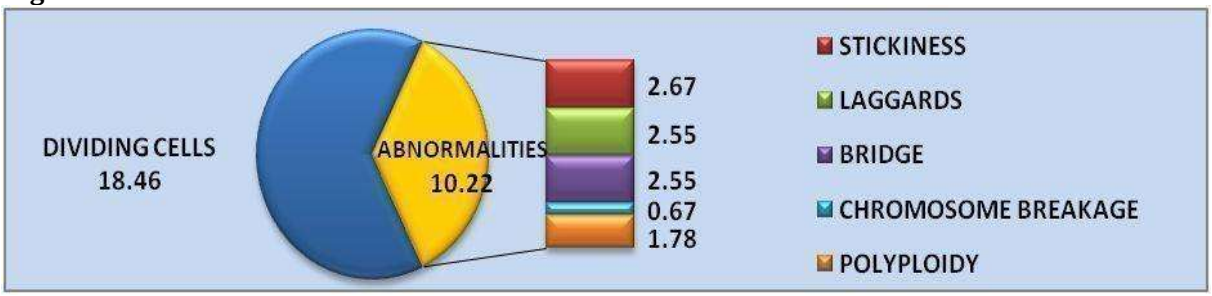

Figure 5: Fruit extract

Plate 2: Mitotic divisions, chromosomal abnormalities and their types induced by 20% concentrations of *Lantana camara* L. extracts in *Vigna mungo* L. var. Vamban-16

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