Mutageneic effects of *Kochia indica* extract on *Vicia faba* L.

Soliman A. Haroun*

Department of Botany, college of Science, Kafrelsheikh University 33511, Egypt
solimanharoun@yahoo.com

Abstract: The present study investigate the effect of *Kochia indica* extract using *Vicia faba* L assay. All parameters investigated were being affected. Four concentrations of 10, 20, 30 and 40% were applied for three time duration (6, 12, 24 h). The percentage of germination and plant height are dose and time dependent, probably due to alterations in cell cycle. Treated roots were highly affected compared to seed treatment. Low doses applied for short time show stimulatory effect on germination, growth and MI parameters. On the other hand high concentrations applied for long time gave rise to substation reduction for all parameters investigated. The percentage of mitotic and meiotic abnormalities increase as the concentration increase and time prolonged. Various types of abnormalities with special reference to micronuclei, laggards and bridges were observed at all concentrations but more frequent at higher doses. Following the same trend meiotic parameters of chromosome association, chiasma frequency and pollen fertility were being negatively affected and dose dependent. High concentrations show low chiasma frequency, pollen fertility and high percentage of abnormalities. Types of multivalents and univalents were also observed. The present studies recommend the use of this extract at concentrations up to 30% to minimize the side effects on plants as non target organism. It also encourages use of botanical extracts for biological control instead of synthetic chemicals. [Journal of American Science 2010;6(7):292-297]. (ISSN: 1545-1003).

Keywords: *kochia indica, Vicia faba*, mitosis, meiosis,chiasma,abnormalities

1. Introduction

Cytogenetic effects of synthetic chemicals used for plant protection have been well documented and previously investigated by many authors (Qureshi *et al*., 1988, Mekki 2008, Vyvyan, 2002, Badr *et al*., 1985, Mohammed, 1986). Almost all studies confirm the harmful effects of synthetic chemicals used in agriculture purposes and increase the environmental pollution which is a global problem. For these reasons many attempts were carried to find out alternative natural compounds for more ecological safety (Alam *et al* 1987, Panda & Sahu 1985, Shehab 1980, Abou elkheir 1992, Haroun & Al shehri 2001, Jose *et al* 2008, Abderrhman 1997, 1998, Adam & Rashad (1984) and Omari, *et al* 1996).

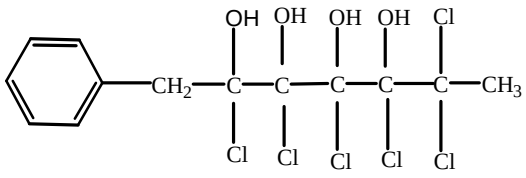
Aqueous extract of *Kochia indica* species (Chenopodiaceae) used in this study has been previously tested by Mahmoud (1998) as pest control. The study shows that the extract could be used as insecticide at certain concentrations, but completely ignores its side effect on crop plants as non target organism. These attract the attention to evaluate the effects of this extract on *Vicia faba* plants.

The aim of this study is to investigate the effect of this extract at different concentrations on the percentage of germination, plant growth (percentage of height of control), mitotic index, mitotic and meiotic aberrations, chromosome association and pollen fertility of *Vicia faba* L plants. This investigation is a part of programme study the potential effect of plant extracts as biological control instead of synthetic chemicals.

2. Material and Methods

Aqueous extract of *kochia indica* (shoot system) was prepared following Nassar (1995) and Mahmoud (1998). 250 g of fresh leaves and stems were ground into 500ml distilled water and left for extraction overnight at room temperature. The extract was filtered using whatman filter paper No1. Further extraction was carried out for more purification.

The elementary analysis of this extract was previously carried out by Mahmoud (1998) using ultraviolet spectroscopy (UV), infrared spectroscopy (IR), gas chromatography (GC) and mass spectrophotometer (MS). The analysis detect the active compound with proposed molecular formula C13 H14 Cl6 O4, molecular weight 447 and chemical structure, 1-phenyl 2,3,4,5,6 hexa chloro 2,3,4,5 tetra hydroxy heptane



1-phenyl 2,3,4,5,6,6 hexa chloro 2,3,4,5 tetra hydroxy heptane.

For mitotic study, two types of treatments were carried out using seed soaking and root treatment experiment. Seeds of *Vicia faba* were presoaked in water for 24 h. and treated with four concentrations 10, 20, 30 and 40 % (same range of concentrations previously used as pesticides by Mahmoud 1998). All concentrations were applied for 6, 12 and 18 h of treatment. Treated seeds were planted in pots half filled with moist sand till germination. Young roots were excised and prepared for mitotic study. Percentage of germination and plant height were recorded at various concentrations relative to control.

For root treatment, fresh roots (1-2 cm) were subjected to the same concentration and time used in seed soaking experiment. Excised roots of both treatments were washed in distilled water and fixed in freshly prepared fixative solution (1:3 v/v acetic alcohol) for 24 hr. Squash technique using feulgen reagent was used for mitotic analysis. Mitotic index and percentage of abnormalities were counted using at least five slides freshly prepared for each concentration.

For meiotic analysis, growing plants (30 days old) were sprayed with the same concentrations applied for mitotic treatments. Young flower were collected before anthesis at early morning (9 am) and fixed in fresh fixative solution. Anthers were macerated in few drops of 2 % acetocarmine to study pollen mother cells (PMC's). Meiotic behavior as chromosome association and chiasma frequency were studied at diaknesis and metaphase I stages. Percentage of meiotic abnormalities and pollen fertility were also studied.

3. Results

The effects of *kochia indica* extract on percentage of germination and plant height (relative to control), mitotic index and percentage of abnormalities were recorded and summarized in table1.

Morphological data represented by percentage of germination and plant height revealed a significance decrease as the concentration increase and the time of treatment prolonged. It is evident from table 1 that low concentrations treatment (10 and 20% for 6hr) show a stimulatory effects compared to control. This result was previously reported by Haroun and Al shehri (2001), Anis and Wani (1997), Lamix and Gupta (1983), Katyayami *et al* (1980) and Abderrhman (1997). In the present study low concentrations induce growth is probably due to chemical components of chloride and phenol groups which might give hormonal effects and enhance plant growth and cell division.

The rest of data show gradual decrease in the percentage of germination and plant height as the concentration increase and the time prolonged recording values of 59.7 and 63.1 at 40% and 24hr for the two parameters respectively. This finding was agree with that previously recorded by Haroun and Al shehri (2001), Abou elkheir (1992), Panda & Sahu (1985) and Alam *et al* (1987).

Mitotic study

Inhibition of mitotic activities was often used for tracing cytotoxic substances. Cytotoxicity was defined as decrease in mitotic index revealed the mitodepressive effect of *Kochia indica* on mitotic index (MI) of *Vicia faba*. The mitotic index of 10 and 20 % concentrations is slightly increased compared to control, insure the relationship between this parameter and morphological characters as previously reported by Abderrhman (1998), Abou elkheir (1992), and Alam *et al* (1987). This increase may due to shorting the duration of mitotic cycle and enhance the interphase cells enter the subsequent division stages (Haroun & Al shehri 2001 and El Kodary 1980) or by induce the synthesis of DNA in dividing cells (Badr *et al* 1985 and Chand & Roy 1981).

The rest of treatments are significantly altered MI gradually as the concentration increased and time prolonged. Significance reduction in MI was recorded in root treatment insure the harmful effects of direct treatments on plants as previously stated by Marant (2003), Pavel & Creanga (2005), Jose *et al* (2008), Haroun and Al shehri (2001) and Banerjee (1992). The lowest values of MI were 2.7 and 2.5 recorded by 40% concentration at 24h for seed and root treatment respectively.

The percentage of mitotic aberrations recorded more or less follow the same trend of mitotic index. With no exception the percentage of aberrations increase as the concentration increase and time prolonged. The highest values recorded for this parameter were 20.7 and 31.9 for seed and root treatment respectively. This trend was previously recorded by Jose *et al* (2008), Pavel & Creanga (2005) and Alam *et al* (1987).

Table 1: Effects of different treatments of *Kochia indica* extract on percentage of germination, plant height (% of control), mitotic index and percentage of abnormalities for seed and root of treated *Vicia faba* L.

treatment	%germ.	% height. of cont.	Mitotic index ± SE		% abnormal. ± SE	
			Seed	root	Seed	root
6h Control	93.1±1.1		11.2±0.11	10.7±0.34	1.5±0.62	1.7±0.25
10	94.3±1.5	105.2	11.6±0.32	10.8±0.41	2.1±0.22	2.7±0.48
20	94.1±1.3	103.1	11.3±0.35	10.2±0.33	3.2±0.17	3.9±0.52
30	75.6±0.9	75.2	9.4 ±0.61	7.5±0.25	6.1±0.31	6.6±0.46
40	71.2±1.3	69.8	8.5±0.51	7.1±0.54	7.5 ±0.22	9.3±0.63
12h control	92.5±0.8		10.5±0.33	10.1±0.25	1.5±0.12	1.6±0.23
10	93.3±1.1	90.7	10.2±0.55	9.3±0.46	2.0 ±0.23	3.1±0.51
20	91.2±1.3	90.5	9.7±0.32	9.2±0.19	4.7±0.41	5.1±0.24
30	71.3±1.4	70.9	8.4±0.51	6.9±0.35	7.8 ±0.71	8.5±0.11
40	68.5±0.9	70.1	8.2±0.57	6.1±0.46	8.3 ±0.55	10.3±0.61
24h control	85.1±0.8		8.1±0.54	7.5±0.44	1.4 ±0.39	1.5±0.60
10	83.2±1.1	83.1	6.4±0.81	6.2±0.39	8.1 ±0.33	9.5±0.91
20	77.6±1.4	74.7	5.1±0.21	4.6±0.11	12.3±0.72	14.7±0.82
30	63.1±1.5	60.5	3.8±0.22	2.9±0.31	15.6±0.53	18.4±0.54
40	59.7±2.0	63.1	2.7±0.50	2.5±0.28	20.7±0.39	31.9±0.87

At higher doses more deleterious effects were observed and reduction in MI. values of 2.7 & 2.5 was recorded for seed and root treatment respectively. The percentage of mitotic abnormalities increased as the concentration increase and the time of treatments prolonged. Values of 31.9 and 20.7 were recorded for root and seed treatment at 40% concentration applied for 24h. This observation was previously stated by Haroun and Al shehri (2001) and Alam *et al* (1987).

Types of micronuclei were observed at various stages of mitosis (interphase, figure1-1; prophase figure1-2; telophase, figure1-4) in both treatments is the manifestation of chromosome damage and disturbance of the mitotic process. The micronucleus are almost acentric fragments, lagging chromosomes or from breakage of chromosomes failed to move to either pole during anaphase of mitosis (Gover and Kaur, 1999, Panda and Sahu, 1985). C-metaphase (figure1-3),C-anaphase (figure1-7), multipolar (figure 1-8), lagging chromosomes (figure1-5, 9), chromosome breaks,(figure1-6), bridges(figure 1-4,9) were observed at different treatments. Recording of some aberrations like lagging, bridges and breaks to some extend insure that the extract have more or less direct effect on chromosomes and spindle fiber apparatus (kong and Ma 1999). Not surprisingly that the percentage of abnormalities recorded is more frequent at high concentrations and root treatment as previously stated by Kabarity & Malallah (1980), Alam *et al (*1987), Jose *et al* (*2*008) and Haroun & Al shehri (2001).

Meiotic study

Number of pollen mother cells (PMC's), percentage of abnormal ones, chromosome association, chiasma frequency and percentage of pollen fertility were recorded and listed in table 2. Meiosis of control plants is normal showing 6.0 bivalents counts 2n = 12 as diploid number. Some abnormalities observed were probably due to squash technique. Value of 1.82 for chiasma frequency was recorded for control sample reflect the normal meiosis and complete pairing between chromosomes. High percentage of pollen fertility is recorded (97.5%) as a penalty of normal meiosis and high chiasma frequency.

Treated plants exhibit various degree of abnormalities more or less depends on concentrations applied. The percentage of abnormalities increase as the concentration increase (table 2). This trend was previously stated by Haroun and Alshehri (2001), Badr *et al* (1985) and Chand and Roy (1981). All treated plants show gradual decrease in chiasma frequencies and pollen fertility as the concentration increase. The lowest value recorded for these parameters are 1.12 and 80.4 at 40% concentration. No dough, there is a strong relationship between the last parameter and percentage of abnormalities and chiasma frequency as it is a penalty of abnormal meiosis and mispairing between chromosomes during meiosis.

For chromosome association, univalents were recorded in frequency ranged between 1.7 - 2.1 at all treatments. It seems to be more or less the material of abnormalities, which consequently affect paring and chiasma frequency. Bivalents association in form of ring type was observed (figure 2-1) and may affect chiasma frequency. The frequency of this parameter decrease as the concentration increase (table 2). Multivalent in forms of trivalent and quadrivalents were observed at diaknesis and metaphase I (figure 2-

1,2) and found more frequent at high concentrations. The formation of odd numbers pairing of chromosomes association as univalents and trivalents probably caused by cryptic structural changes in chromosomes forming genetic differences which restrict paring with other homologous as previously stated by Anis and Wani (1997) and Haroun and Al shehri (2001).

Some meiotic abnormalities were observed insure the harmful effects of this extract especially at high concentrations. Types of stickiness at metaphase I (figure 2-3), disturbed anaphase 11(figure 2-6) bridges at anaphase I and II (figure 2- 4,5) were observed and recorded more frequent at high doses following the same trend in mitotic study.

No dough meiotic disorders affect normal disjunction, pairing and chiasma frequency and subsequently affect crossing over which is the site of exchange genetic material during meiosis. This finaly lead to form abnormal and non functional gametes which can not compete any pollination or sexual reproduction as previously stated by Ramanna (1974) and Haroun and Al shehri (2001). Although the mechanism of mitotic and meiotic disturbance of this extract is not clear the possibility of genetic instability is insured especially at high doses.

The concentration of of 40% applied in the present study was previously recommended by Mahmoud (1998) as efficient potential insecticide and best control. The present data confirm the harmful effects of this concentration on plants as non target organism and recommend use of lower concentrations (ranged between 20-30%) which show low harmful effects on plants and significantly control insect fertility and reproduction.

Despite this extract is more or less chemical compound, but it is much safer than synthetic pesticides and highly recommended for biological control.

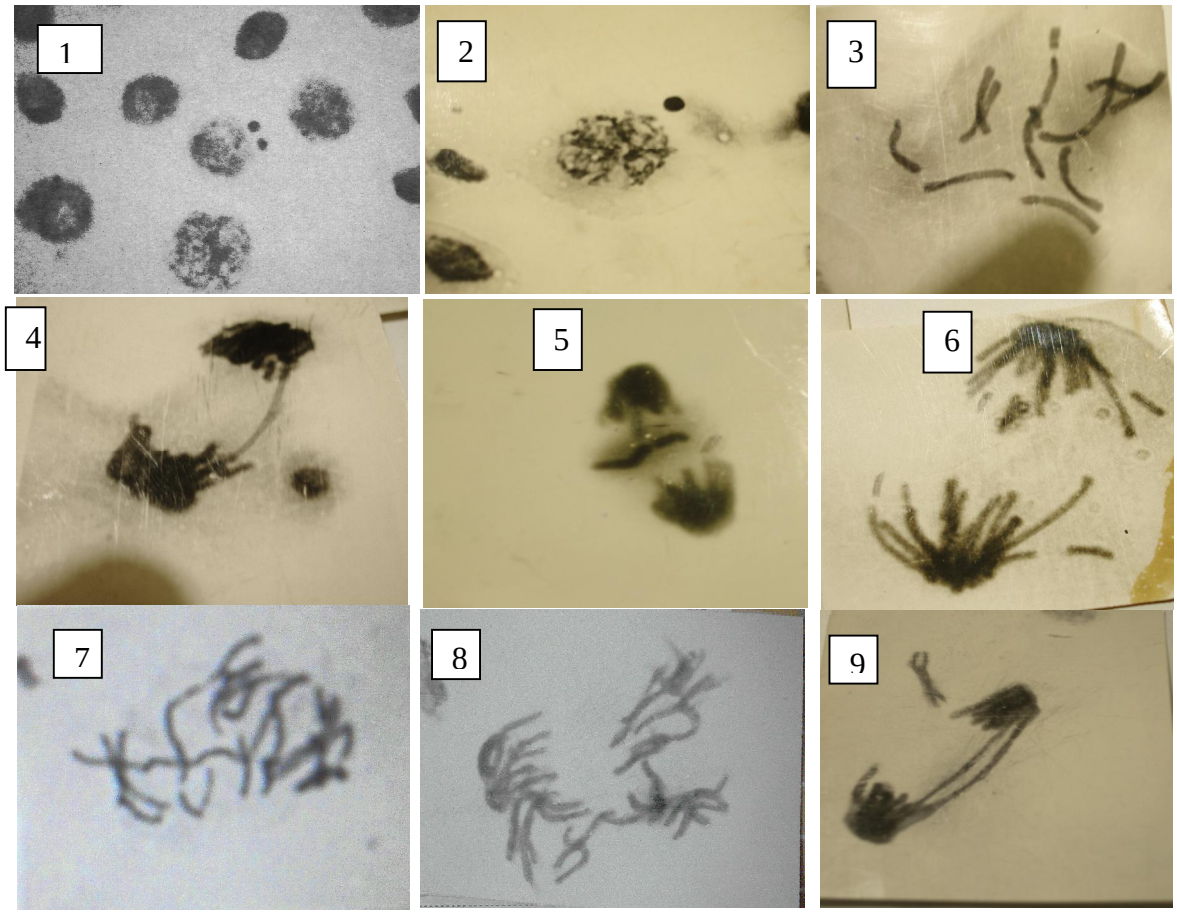


Figure 1: 1, miconuclei at interphase (x1000). 2, micronuclei at prophase (x1400). 3, c-metaphase (x1600). 4, micronuclei and bridge at telophase (x 1600). 5, lagging at telophase (x 1600). 6, breakes at anaphase (x 1600). 7, c-anaphase (x1600). 8, multipolar (x1600). 9, bridges and lagging (x1600).

Table 2: Effects of different concentrations of *Kochia indica* extract on percentage of abnormal pollen mother cells (PMC's), chromosome association, chiasma frequency and percentage of pollen fertility of *Vicia faba* L.

Treatment %	No.PMC,s examined	%abnorm. ±SE	Chromosome association I	II	III	IV	Chiasma frequency	%poll. Fert. ±SE
Contol	71	0.9±0.12	0.0	6.0	0.0	0.0	1.82	97.1±1.21
10	87	2.6±0.22	1.8	5.2	0.0	0.0	1.71	93.4±0.99
20	79	4.1± 0.31	2.1	4.8	0.0	0.0	1.53	89.3±1.10
30	68	4.5± 0.45	1.7	2.9	0.21	0.96	1.41	85.1±1.12
40	81	5.3± 0.33	1.8	2.7	1.91	0.22	1.12	80.4±1.39

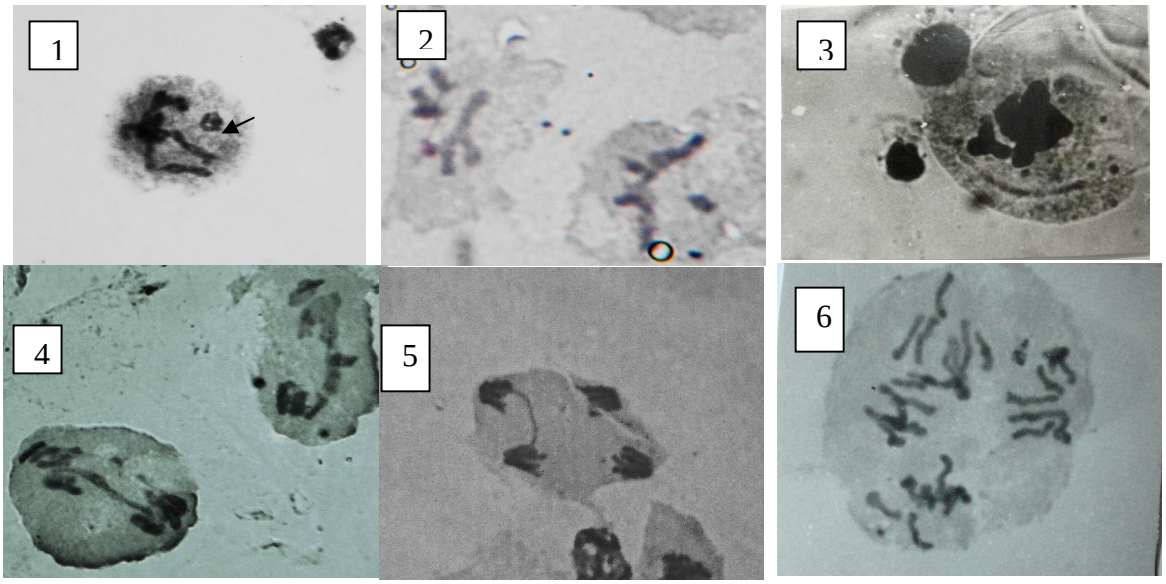


Figure 4: diplotene with ring bivalent arrow(1), diaknesis with multivalents (2), sticky metaphase I (3), bridges at anaphase I, and telophase II (4,5) , disturbed anaphase II (6) (x1600)

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