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Cyto-physiological Effects of Aqueous Extracts of Some Weeds and Clove on the Growth of Chinese Faba Bean (*Vicia faba* L.)

Mahmoud F. Moustafa[1,2*], Sulaiman A. Alrumman[1], Hoida Zaki[2], Naglaa Loutfy[2] and Mohamed Hashem[1,3]

[1]Department of Biology, Faculty of Science, King Khalid University, Abha, Saudi Arabia.
[2]Department of Botany, Faculty of Science, South Valley University, Qena, Egypt.
[3]Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Author MFM designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors SAA, HZ and NL managed the analyses of the study. Author MH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Cytological and physiological effects resulted from aqueous extracts of *Aster squamatus, Euphorbia terracina, Euphorbia peplus*, *Syzygium aromaticum*, *Acacia pycnantha*, *Aloe vera*, *Euryops arabicus* and *Tamarix aphylla* to the growth of Chinese faba bean (*Vicia faba* L.) seedlings were evaluated. Examined growth parameters including seeds germination percentage and the fresh and dry weights of root and shoot and their length greatly affected by types of extracts during the time course of germination. Among all tested extracts the *Syzygium aromaticum* (Clove) and *Euphorbia peplus* extracts found to be mitodepressant and induced noticeable frequencies of traditional chromosome aberration. Those two extracts also caused significant decreases to the length, fresh and dry weights of root and shoot of Chinese faba bean seedling. *Euphorbia terracina*

**Corresponding author: E-mail: mfmostfa@kku.edu.sa;*

and *Tamarix aphylla* extracts caused complete success of seed germination while *Aster squamatus,* caused noticed stimulation in root length, root fresh weigh (mg/plant) and root dry followed by *Euphorbia terracina*. *Euryops arabicus* caused significant stimulation in shoot length, shoot fresh weight, root lenght and in root fresh and dry weight followed by *Tamarix aphylla*.

Keywords: Aster squamatus; Euphorbia terracina; Euphorbia peplus; Syzygium aromaticum; Acacia pycnantha; Aloe vera; Euryops arabicus; Tamarix aphylla.

1. INTRODUCTION

Weeds plant or invasive species are considered unwanted plants that compete with native and cultivated plants for various resources including nutrient, light and water, etc. However, some weeds extracts or their ingredients were found to have an effect on cell division, rate of chromosomal aberration, growth parameters of some plants or can be used as an organic fertilizer [1,2,3]. The analysis of chromosomal deformations occurring over a cell cycle due to exposed to plant extracts is one of the few indirect methods to evaluate damages in cell systems. For this purpose, there are *Vicia faba* and *Allium cepa* chromosome deformation test [4,5]. The inhibition in cell division by chromosomes, mitotic index, amount of DNA in the nucleus and replication index usually are used as indicators of cytotoxic and genotoxic effects. As a strange substances introduced to the cells can cause an increase in the chromosomal aberrations (CA), damage to enzymes, membranes, proteins, and DNA resulted in cellular damage and toxicity [6]. From the medical point of view, the traditional medicine, especially in developing countries many weeds plants are frequently used that escaped toxicity test before marked for sale as drugs. Yet several reports revealed that some medicine of plant origin may contains toxic substances, for example, the death following ingestion of herbal medicine, hepatic failure, mutagenicity, clastogenicity and carcinogenicity to human after treatment with herbal medicine [7,8]. From the standpoint of economic plant, aqueous extracts of some weeds plant as for example*, Amaranthus hybridus*, *Parthenium hysterophorus*, *Datura stramonium* and *Argemone mexicana* leaf [9], compost of *Parthenium hysterophorus* [10], aqueous extracts of different part of *Eclipta alba* [11], etc, showed to have allelopathic effect on the growth of seedling crops. To the best of our knowledge, few scientific reports on the cytological and allelopathic effect of Saudi Arabia weed species to the germination and to the growth of economically important cultivated plants such as *Vicia faba* L. Therefore, the objective of this research is to determine cytological and allelopathic effects that induced by aqueous extracts of *Aster squamatus, Euphorbia terracina, Euphorbia peplus*, *Syzygium aromaticum* (Clove), *Acacia pycnantha*, *Aloe vera*, *Euryops arabicus* and *Tamarix aphylla* in the root tips of Chinese faba bean (*Vicia faba* L.).

2. MATERIALS AND METHODS

2.1 Sample Collection

Plant materials including the leaves of *Aster squamatus* (Spreng.) Hieron., *Euryops arabicus* Steud. ex Jaub. & Spach, (Family – Asteraceae), *Euphorbia terracina* L.*, Euphorbia peplus* L., (Family – Euphorbiaceae), *Acacia pycnantha* Benth., (Family – Fabaceae), *Aloe vera* (L.) Burm. f., (Family – Xanthorrhoeaceae) and *Tamarix aphylla (L.) H.Karst (*Family - Tamaricaceae) were collected from different localities of ABHA, Asir province, Kingdom of Saudi Arabia. Plants were identified based on the vegetative and floral characteristics throughout taxonomists in the Biology Department, Faculty of Science, King Khalid University [12]. Fruits of *Syzygium aromaticum* (L.) Merr. & L. M. Perry (Family- Myrtaceae) were collected from the market and investigated parts were washed thoroughly with distilled water and allowed to dry in room temperature for further use.

2.2 Extracts Preparations

Washed fresh parts from each plant were grinded directly for 20 minutes, and then filtered through Whatman no. 4. The concentrations of the extracts were expressed as percentage of fresh weights of plants in water [13].

2.3 Treatment Materials

Seeds of Chinese faba bean (*Vicia faba* L.) were washed thoroughly with distilled water and in petri plates treated with eleven percent of aqueous plant extracts of *Aster squamatus*,

Euphorbia terracina, *Euphorbia peplus*, *Acacia pycnantha*, *Aloe vera*, *Euryops arabicus, Tamarix aphylla* and fruits of *Syzygium aromaticum* and kept sunken in extracts for 15 days as shown in (Table 1). As a positive control some seeds grown in distilled water under the same conditions and for each treatment seven seeds were used in three replications. According to Darlington and La-cour [14], with little modification the cytological treatments were carried out by using Faulgen's Squash technique and chromosome aberration were observed with light microscopy (LM). By examining about 371 metaphases from seven root tips per preparations, the types and frequency of chromosome aberration were determined, and chromosome abnormality were recorded. Mitotic activity was estimated as mitotic index (MI) by scoring dividing cells to the total number of cells examined and chromosomal aberrations at metaphase, anaphase and telophase were determined. Cytological effect of investigated plants was evaluated as the percentage of cells showing chromosomal abnormalities [15,16].

Germination rates were determined on the 15th day (Number of germinated seeds/Number of total seeds × 100), whereas a seed was considered to have a germination in case a radical reached up to a length to 1 mm. On the 15th days after germination, root and shoot length of *Vicia faba* treated with various plant extract were measured and then dried rapidly in an oven at 80°C to determine the dry weight as mean of 7 plants (DW) [17]. Data was statistically analyzed using SPSS software (version 12), by applying Tukey post-hoc test to compare statistical significance of differences among the mean values.

3. RESULTS AND DISCUSSION

3.1 Chromosome Deformations

Exposure of Chinese faba bean (*Vicia faba* L.) to eleven percent aqueous extract of *E. terracina*, *E. peplus*, *S. aromaticum, A. pycnantha*, *A. vera*, *A. squamatus, E. arabicus* and *T. aphylla* produced chromosome abnormalities as shown in (Fig. 1 and Table 1). These extract induced chromosome abnormalities ranging from 2.20% to 5.26% on root tips of Chinese faba bean (*Vicia faba* L.). It is noticed that there were correlation between the types of plant extracts used and the percentage of affected cells. The highest frequencies of chromosomal aberrations was obtained from aqueous extract of *S. aromaticum* having a values (5.26%) followed by *E. peplus* (4.85%). The majority of chromosomal abnormalities in the root tip cell treated with aqueous extract from *S. aromaticum* and *E. peplus* were disturbed anaphase, anaphase with lagging chromosome, sticky anaphase, disturbed metaphase, break in chromosome, bridge and lagging chromosome in anaphase, diagonal metaphase, break in metaphase and rest in interphase. In the case of *A. vera* aqueous extract treatment, there was higher frequencies of chromosomal aberrations (4.29%) than other weeds and for negative control. The majority of chromosomal abnormalities were sticky metaphase, disturbed anaphase, disturbed metaphase, rest in interphase and vacuolated interphase. Treatments the roots tips with aqueous extract of *E. terracina* and *A. pycnantha*, the percentage of chromosomal abnormalities were found to be almost the same having values of 3.35% and 3.27% respectively. The majority of chromosomal abnormalities were irregular prophase, sticky metaphase, disturbed anaphase, anaphase with lagging chromosome, non-congressional chromosome in metaphase, disturbed metaphase, bridge and/or lagging chromosome in anaphase, rest in interphase and diagonal metaphase. There was less numbers of chromosomal aberrations in root tips by applying aqueous extracts of *A. squamatus* and *E. arabicus* recording values of 2.91% and 1.96% respectively. The majority of chromosomal abnormalities were irregular prophase and sticky metaphase. Therefore, the occurrence of chromosomal aberrations in root tips of *Vicia faba* plants varied greatly among the type of plant extracts used. The natural of chromosomal abnormalities found in our study had been reported by many investigators as for example Sobita and Bhagirath [18] reported that *Nerium odorum* and *Solanum indicum* were mitodepressant and induced higher frequencies of traditional chromosome aberration than other plant extracts obtained from *Andrographis peniculata, Nyctanthes arbortristis, Phlogacanthus thyrsiflorus* and *Kaempferia galangal* plants. The methanolic extracts of *Euphorbia hirta* were tested on root meristems of *Allium cepa* found that it caused chromosomal abnormalities including stickiness, c-mitosis, bridges, vagrant chromosomes and micronucleated cells at interphase [19]. How these plants extracts might affect the chromosomes of Chinese faba bean? It was reported that the inducing the chromosomes abnormalities probably due to the blockage of DNA synthesis or inhibition of spindle formation

[20]. Christopher and Kapoor [21] suggested that the reduction of the mitotic index due to the obstruction of the onset of prophase, the arrest of one or more mitotic phases, or the inhibition in the rate of cell progression through mitosis. Darlington and Mc-Leich [22] and La-Cour and Rutishausar [23] attributed this abnormality to the breaking down or depolymerization of chromatin threads that made the chromosome have a sticky surface. Hsu et al. [24] and McGill et al. [25] speculated that chromosome stickiness might be caused by more influx or an abnormal activities of the RNA-containing particles which cover the metaphase and anaphase chromosomes or due to physical interferences with chromosome condensation.

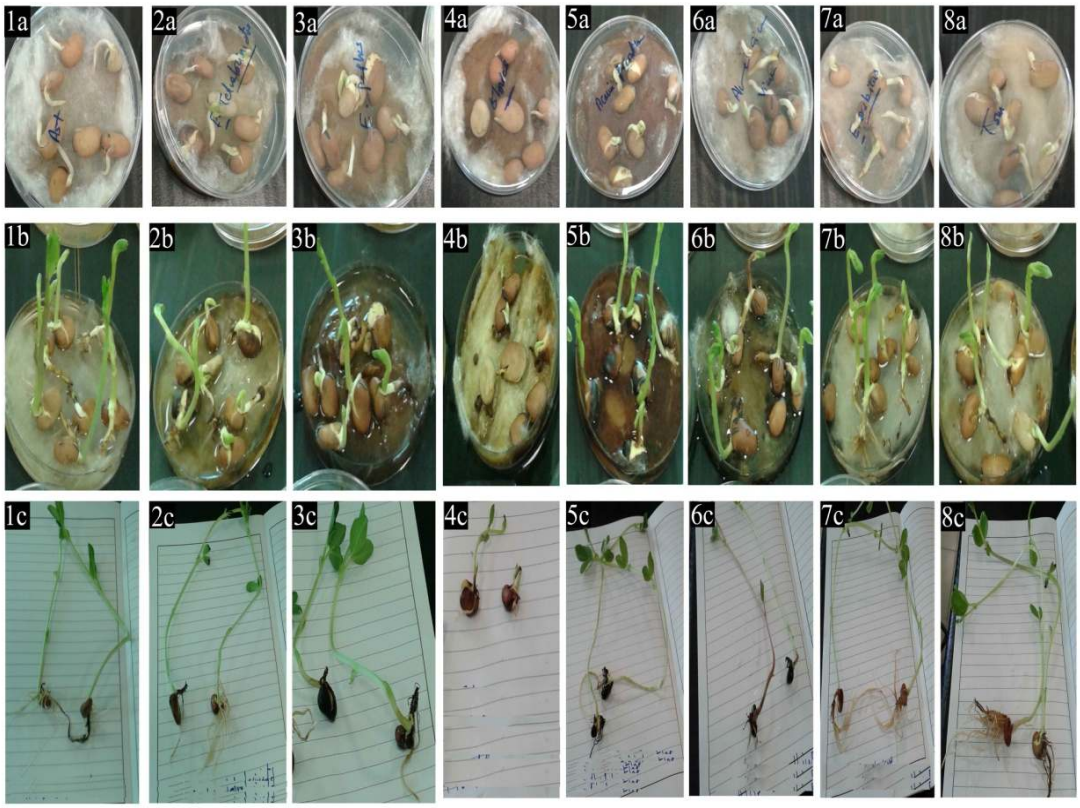

Fig. 1. Physiological impact of aqueous weeds extracts on some growth parameters of Chinese faba bean (*Vicia faba* L.). (a, b, c, represents germination times, a = one day; b = 3 days; c = 15 days); 1-Water; 2= *E. terracina*; 3= *E. peplus*; 4= *S. aromaticum; 5= A. pycnantha*; 6= *A. vera; 7= E. arabicus;* 8= *T. aphylla*

Table 1. Chromosome abnormalities percentage in Chinese faba bean (*Vicia faba* L.) as results of treatment with 11% aqueous weed extracts

Treatment	Concentrations	Numbers of cells examined	Cells having chromosome abnormalities	
			Number	%
Water	11%	409±1.25	1.00±0.01	0.24
A. squamatus	11%	309±1.87	9.00±0.05*	2.91
E. terracina	11%	387±0.98	13.0±0.26	3.35
E. peplus	11%	350±2.65	17.0±0.98	4.85
S. aromaticum	11%	361±3.67	19.0±0.29	5.26
A. pycnantha	11%	366±3.68	12.0±0.21*	3.27
A. vera	11%	349±.0.25	15.0±0.65	4.29
E. arabicus	11%	407±4.25	8.00±0.98	1.96
T. aphylla	11%	409±3.98	9.00±0.21*	2.20

The percentage significant compared to control (*) at P ≥ 0.05 level

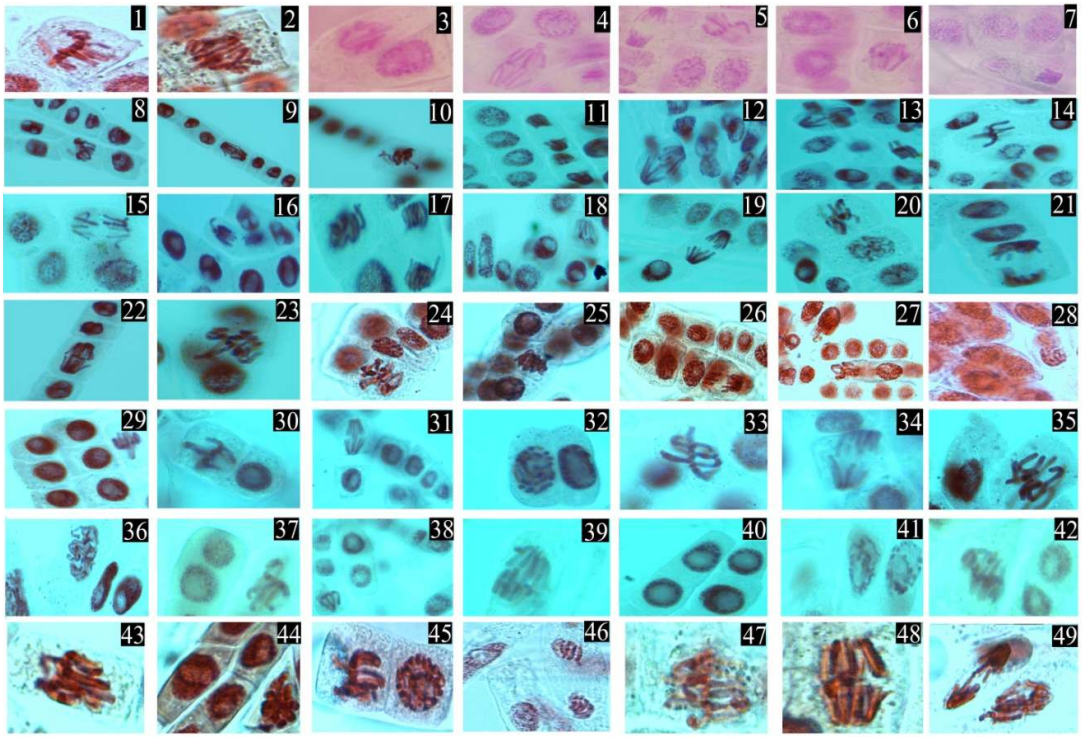

Fig. 2. Chromosomal aberrations of Chinese faba bean (*Vicia faba* L.) upon the effect of aqueous weeds extracts. *A. squamatus* (1-2); *E. terracina* (3-6); *E. peplus* (7-23); *S. aromaticum* (24-28); *A. pycnantha* (29-36); *A. vera* (37-42); *E. arabicus* (43-44); *T. aphylla* (45-49). Chromosome abnormalities as followings: Irregular prophase, Fig. (1 and 36). Sticky metaphase, Fig. (2, 29, 42, 43, 44, 45 and 47). Disturbed anaphase, Fig. (3, 6, 10, 18 and 39). Anaphase with lagging chromosome, Fig. (4, 9, 22 and 26). Non-congressional chromosome in metaphase (Fig.5). Sticky anaphase, Fig. (7 and 48). Disturbed metaphase, Fig. (8, 20, 23, 24, 25, 30, 35, 37 and 38). Break in chromosome and/or sticky anaphase, Fig.(11, 15, 16, 19, 21, 49). Bridge and/or lagging chromosome in anaphase, Fig. (12, 13, 31). Diagonal metaphase (Fig.14). Lagging chromosome in teleophase, Fig. (17). Break in metaphase, Fig.(27). Rest in interphase, Fig. (28, 32, 33 and 41). Diagonal metaphase, Fig. (34). Vacuolated interphase, Fig. (40). Diagonal teleophase, Fig. (46)

Table 2. Effects of aqueous weeds extracts on shoot and root length (cm), fresh and dry weights of root and shoot (mg/plant) and germination percentage in 15-days old seedlings of Chinese faba bean (*Vicia faba* L.)

Seeds irrigated by water and by aqueous extract	Shoot length (cm)	Shoot fresh weigh (mg/plant)	Shoot dry weight (mg/plant)	Root length	Root fresh weigh (mg/plant)	Root dry weight (mg/plant)	Germination %
Water	9.49±1.55	8.60±1.10	0.76±0.04	6.67±1.15	1.10±0.42	0.14±0.06	100
A. squamatus	12.7±0.86	6.91±1.61	1.10±0.04	6.33±1.11	1.04±0.40	0.13±0.05	85.7
E. terracina	9.77±1.92	8.22±1.12	0.69±0.20	2.66±1.48	0.81±0.31	0.10±0.04	100
E. peplus	4.75±1.03	3.93±0.51	0.66±0.02	0.46±0.11	0.18±0.07	0.02±0.01	42.9
S. aromaticum	1.19±0.17	3.33±0.61	0.56±0.02	0.41±0.08	0.08±0.03	0.01±0.00	85.7
A. pycnantha	9.66±1.4	7.02±1.10	1.43±0.15	1.55±0.35	0.39±0.15	0.05±0.02	85.7
A. vera	6.86±0.2	4.43±0.73	0.70±0.01	1.28±0.59	0.39±0.15	0.05±0.02	85.7
E. arabicus	13.9±0.9	10.64±1.91	0.88±0.03	2.66±1.49	0.80±0.30	0.10±0.05	85.7
T. aphylla	12.0±1.7	12.43±1.88	1.14±0.08	1.86±0.76	0.49±0.18	0.06±0.03	100

3.2 Allelopathic Effects

Water leaves extract from *Aster squamatus*, *Euphorbia terracina*, *Euphorbia peplus*, *Acacia pycnantha*, *Aloe vera*, *Euryops arabicus and Tamarix aphylla* and fruits extract of *Syzygium aromaticum* exhibited variable effect on the number of germinated seeds of Chinese faba bean (*Vicia faba* L.) as compared to the control (Table 2). *Euphorbia terracina* and *Tamarix aphylla* extract caused complete success of seed germination of Chinese faba bean as compared by control (100%). Extract gained from *Aster squamatus*, *Syzygium aromaticum*, *Acacia pycnantha*, *Aloe vera* and *Euryops arabicus* were found to have the same effect on the rate of seed germination (85.7%). The growth rate of shoot length (cm), shoot fresh weight (mg/plant) and shoot dry weight (mg/plant) of *Vicia faba* were also affected by the treatment of various weed extracts (Table 1). In case of *A. squamatus, E. arabicus* and *T. aphylla* caused the highest effect followed by *E. terracina* and *A. pycnantha* than control and other tested weeds species after 15 days of germination. However, *S. aromaticum* caused severe inhibitory. Effect of tested weed extracts on root length, root fresh weigh (mg/plant) and root dry weight (mg/plant) of *Vicia faba* plant varied also greatly. *A. squamatus* extract caused significant stimulation in root length, root fresh weigh (mg/plant) and root dry weight of Chinese faba bean followed by *E. terracina* as compared by control (100%). *A. pycnantha* caused a moderate effect on the faba bean roots various parameters while *S. aromaticum and E. peplus* caused severe inhibitory effect (Fig. 1 and Table 2). Therefore, there are an association between chromosome abnormalities and the studied growth parameters of *Vicia faba* plant. Previous studies showed that strange excaudate to the plant roots could effect on germination percentage, seedling growth and mitotic cell division [26]. A lot of research has been carried out about the allelopathic effect of weeds extract on the plant growth. For example, remarkable effects in seed germination of *Solanum* sp. and *Capsicum* sp. by allelopathic effect of leachates of *Parthenium* sp. [27]. Naeem et al. [28], showed a profound effect of *Parthenium hysterophorus* root, stem and leaf extracts on the germination rate and seedling growth of wheat cultivar. There were stimulatory effect on seed germination, root-shoot length and seedling growth of *Sorghum vulgare* Pers. by using aqueous extract of *Portulaca oleracea* L. [29]. Bhadha et al. [30] showed that extracts of *P. stratiotes* significantly increased root length of rice seeds when applied at a rate of 1 g/petri dish. Allelopathic potentials of *Citrullus colocynthis* shoot system using different concentrations (25, 50, 75 and 100%) showed that there was significant decline in germination of *Hordeum vulgare* but *Vicia faba* not affected [31].

4. CONCLUSION

The present research provides the evidence of allelopathic potential and cytological effects of aqueous extracts of *Aster squamatus*, *Euphorbia terracina*, *Euphorbia peplus*, *Acacia pycnantha*, *Aloe vera*, *Euryops arabicus, Tamarix aphylla* and fruits of *Syzygium aromaticum* to the *Vicia faba* plants. However, this allelopathic activity of this weeds plant may be having a different effect in the field or by applying different concentrations. Therefore, further studies should be done to confirm such activities. There were obviously strong relationships between various growth parameter and chromosome abnormalities in the root tips of *Vicia faba*.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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